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AUSTRALASIAN ANNALS OF MEDICINE

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OBSERVATIONS ON KURU: I. A POSSIBLE GENETIC BASIS[1]

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SUMMARY

Kuru, a recently described neurological disease, occurs with high frequency among the Fore people, in a remote part of the Eastern Highlands of New Guinea.

The results are given of a genealogical survey carried out in the northern Fore area. The survey recorded 2099 individuals, of whom 913 were dead. The cause of death was given as kuru in 204 instances, including 177 females (21 children) and 27 males (19 children).

An analysis is presented which shows that male victims of kuru are born almost exclusively to mothers with the disease, and that there is a similar association between young female victims and their mothers. The incidence of the disease is almost equal in males and young females, while a larger proportion of adult females is affected.

A tentative genetical hypothesis is advanced, suggesting that kuru may be controlled by a single autosomal gene " K ", which is recessive to its allelomorph " k " in males, and dominant in females. The females of genotype KK tend to have an early onset of the disease, while those of genotype Kk tend to have an onset in adult life.

THE recent description by Zigas and Gajdusek (1957) of the disease " kuru " amongst the Fore tribe of the Eastern Highlands of New Guinea has attracted much interest throughout the world because of many features which are remarkable and some which are possibly unique.

Extensive investigations of many possible environmental factors have been made with entirely negative results so far (Gajdusek and Zigas, 1957, 1958). The possibility that the disease has a genetical basis has already been suggested by these authors, but no detailed pedigree study has been recorded by them. Accordingly, an attempt was made during a recent expedition to the Fore area to collect genealogical data. The present article describes the collection, analysis and interpretation of this material. Clinical and pathological studies made at the same time will be reported in following articles (Simpson, Lander and Robson, 1958; Fowler and Robertson, in the press).

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GENERAL REMARKS

The Fore people are a linguistic group of some 10,000 inhabiting an area of about 500 square miles of isolated rugged country to the east of Mount Michael in the Eastern Highlands. To the south their territory is bounded by the Lamari river and the country of the warlike Kukukuku people. To the east are the neighbouring tribes of Kimi, Keiagana and Iate. To the north are the Usurufa and Kanite, while the tribes adjoining the Fore to the westwards are the Awa and the Auiana (Figure I). It is reported that kuru occurs only among the Fore and in closely adjoining groups with a tradition of intermarriage with them (Gajdusek and Zigas, 1958).

The Fore people have only recently come in contact with Europeans. An Administration patrol post was established at Okapa in 1954. R. M. Berndt and his wife were in the northern part of the area in 1951–53. They noted the condition of kuru and described the sorceries associated with the disorder, which they regarded as being psychosomatic in origin (Berndt, 1958). V. Zigas, the Administration Medical Officer stationed at Kainantu, was the first to call medical attention to the condition in 1955.

The Fore people themselves believe that kuru is induced by sorcery, and a well defined and established ritual is recognized (Berndt, 1958). The close relatives of a kuru victim take steps to identify the individual thought to have been the sorcerer, who is then murdered according to the ritual known as Tokabu. The Fore people are known and feared by neighbouring tribes over quite a large area on account of their reputation as sorcerers, and the area over which this has spread may be some indication that kuru has existed for a considerable time.

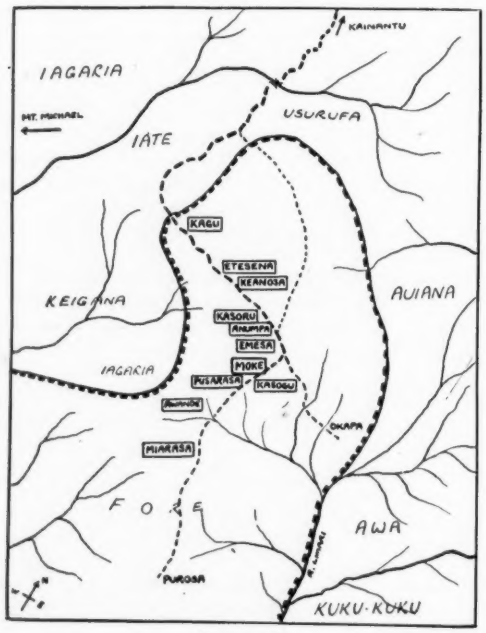

FIGURE I

North Fore area, showing villages included in survey

The "village" or group of small hamlets appears to be the social and political unit of Fore society. Until very recently much small-scale but continuous inter-village warfare occurred. Freedom of movement of any individual within the tribal area of the Fore would depend on the political relations of the various villages at that particular moment. There is no hint of any major movement of Fore people outside the present tribal area within recent history. Some intermarriage has occurred in border areas between the Fore and the Kimi, Keiagana, Kanite, Iate and Usurufa tribes, but not with the Kukukuku, Awa or Auiana people (Gajdusek and Zigas, 1958).

The Fore people are polygamous, the bride-price commonly being paid in pigs. Wives are usually obtained from within the same village but may, on occasion, be sought in some neighbouring "friendly" village. According to Berndt (1954), first cousin marriages are preferred, but the present authors have found little evidence of this. Commonly, however, a man will marry a sister of a deceased wife, and conversely, on the death of her husband, a woman will join the household of one of his brothers. Divorce occurs, usually because of childlessness. Promiscuity is not evident. Adoption of children is a very common practice, those adopted then being regarded as brothers and sisters of existing children of the adopting couple. However, blood relationships, though not regarded as important, are not forgotten.

METHODS.

The data on which the present study is based were collected by two of us (F. A. R. and H. N. R.) during January, 1958. The main part of these data came from a genealogical survey conducted in the northern Fore area, and this will be referred to as the Moke-Kagu or "R" file. Two other collections of data will be referred to as the "K" file and the "Z" file.

The Moke-Kagu or 'R' File.—For this purpose a number of "villages" were selected, namely Moke, Kasogu, Anumpa, Kasoru, Etesena, Keanosa and Kagu (Figure I). These "villages" are situated roughly along a line running from the patrol post at Okapa towards the northern Fore tribal boundary. An attempt was then made to record the family trees of as many as possible of the inhabitants of these villages, without any specific reference to kuru. Communication was achieved through a pidgin-speaking Fore native. The starting point was usually a group of older men and women, each of whom was questioned in turn. Genealogies were extended as far as the memories of informants permitted. Repeated visits and the independent questioning of different members of the same family enabled a good deal of cross-checking of information to be done. In each instance the sex, birth order and approximate age were recorded. In the case of deceased individuals age was estimated by an indication of height in the case of children and by similarity to known living individuals in the case of adults. The cause of death was recorded as given by our informants. Fortunately for our purpose, kuru, with its distinctive clinical picture, is readily and accurately recognized by the Fore people. We were much impressed by the apparent accuracy and consistency of the information supplied.

"K" File.—A group of 10 pedigrees was constructed as the result of specific enquiry from the close relatives of kuru victims who were actually seen during our visit to the area.

"Z" File.—The family histories of 63 observed victims of kuru were generously made available by Dr. V. Zigas. The majority of these victims came from areas other than those covered in the Moke-Kagu survey.

This communication deals mainly with an analysis of the contents of the "R" file. It is hoped to present later a more extended study of the whole material.

RESULTS

The "R" file contained records of 2099 individuals, as shown in Table I. Of these, 1186 were alive at the time of the survey, 670 being male (440 adults) and 516 female (312 adults). In presenting these results, individuals whose estimated age was 17 years or more have been classified as "adult". This division suggested itself after study of the age distribution in 193 current cases (the records of which were kindly supplied by Zigas and Gajdusek, 1958) and after comparison of this with our own data. A histogram for the age distribution in both sexes for the above 193 cases is shown in Figure II. The age distribution for females, which is bimodal, may be considered as the sum of two unimodal distributions, one for young females and the other for adults. As may be seen from the histogram, the age of 17 years comes close to separating these two groups.

Of the 913 deaths recorded in the survey, kuru was given as the cause in 204 instances and tokabu in 88 cases, 547 individuals died from other causes, while in 74 the cause of death was unknown.

The shortage of living females, especially adults, which is shown in the above figures, would seem to reflect the effects of kuru, which was responsible for the deaths of 177 females,

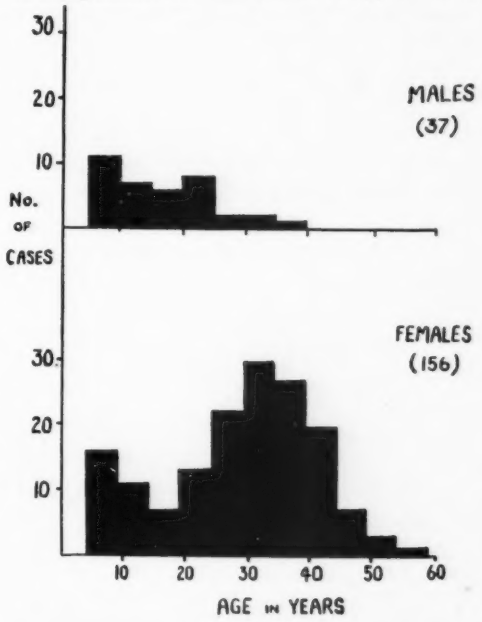

FIGURE II

Histogram of age and sex distribution of the subjects in 193 observed cases of Kuru (Zigas and Gajdusek, 1958)

as compared with only 27 males. On the other hand, 322 males died from other causes, as against only 225 females.

It may be noted that, of the deaths from causes other than kuru or tokabu, about one-

TABLE I.

Individuals Recorded in the "R" File

Sex	Age	Alive	Dead				Totals
			Kuru	Tokabu	Other Cause	Cause Unknown	
M.	Adult	440	8	47	248	32	775
	Young	230	19	4	74	2	329
	Total	670	27	51	322	34	1104
F.	Adult	312	156	34	171	36	709
	Young	204	21	3	54	4	286
	Total	516	177	37	225	40	995
Grand totals		1186	204	88	547	74	2099

quarter (74 out of 322 males and 54 out of 225 females) occurred in those under 17 years of age. Because of this high childhood mortality rate, a not inconsiderable number of potential kuru victims must die from other causes before kuru develops.

Sex and Age Distribution of Kuru

Table II gives the sex and age grouping of the subjects in the 204 reported deaths from kuru in the "R" file. Table II also shows, for comparison, the sex and age grouping of the subjects in the 193 current cases of kuru studied by Zigas and Gajdusek (1958) and also the 20 cases of kuru included in the "K" file. It may be seen that there is remarkably good agreement between these very differently obtained series. This may help to allay somewhat the very reasonable doubt as to the reliability of the present survey, which was dependent on the accuracy of native diagnosis and memory. Each of the series in Table II shows the same preponderance of adult females, and it may also be noted that the numbers of young females dying of kuru approximates very closely to the total of adult and young males involved.

TABLE II.

Sex and Age Distribution of Kuru Cases.

Series	Age	Females	Males
"R" File (204 cases)	Adult ..	156 (77%)	8 (4%)
	Young ..	21 (10%)	19 (9%)
		177 (87%)	27 (13%)
Zigas and Gajdusek (1958) (193 cases)	Adult ..	122 (63%)	16 (8%)
	Young ..	34 (19%)	21 (11%)
		156 (81%)	37 (19%)
"K" File (20 cases)	Adult ..	12 (60%)	1 (5%)
	Young ..	4 (20%)	3 (15%)
		16 (80%)	4 (20%)

Adult Sibships

If kuru makes a greater relative contribution to the death rate in adult than in young females, the proportion of female deaths from kuru should be larger in adult than in young families. To test this, records of adult sibships in which all living members had reached the age of 17 years were abstracted from the "R" file. These are shown in Table III. It will be seen that there is a considerable reduction in the number of surviving females, there being only 46 out of 198 (23%), as compared with 102 males out of 227 (45%).

Deaths of Offspring of Males Dying of Kuru

The age of onset of kuru in males and the late age of marriage of Fore men make it unusual for a male kuru victim to have children. Only four such examples were encountered in the "R" file. (In three of these instances the wife also died of kuru.) Of the six offspring of these four marriages, two females and one male died in infancy; while one girl aged five and two boys aged six and seven years respectively were alive at the time of the record.

Deaths of Offspring of Women Dying of Kuru

Table IV shows the recorded causes of death, in the "R" file, of offspring whose mothers died of kuru (Group A), as compared with those whose mothers died of tokabu (Group B) or from other causes (Group C). (The members of the three families in which both parents died of kuru have not been included in Table IV.) A number of interesting points emerge. A comparison of Groups A and C reveals that kuru caused the death of two-thirds (18/27) of the adult daughters of women who died of kuru, as compared with less than half (45/95) of the adult daughters of women who died from other causes. It may also be noted that 6 of the 24 kuru deaths in Group A occurred in young girls, this being

TABLE III.

Total Numbers of Members of Adult Sibships ("R" File)

Sex	Age	Alive	Dead				Totals
			Kuru	Other Cause	Tokabu	Not Known	
M.	Adult	102	5	73	18	10	208
	Young	—	8	10	1	—	19
	Total	102	13	83	19	10	227
F.	Adult	46	75	38	7	10	176
	Young	—	6	15	1	—	22
	Total	46	81	53	8	10	198

TABLE IV.

Causes of Death Among Offspring ("R" File)

Cause of Death of Mother	Age at Death	Deaths of Offspring					
		Female			Male		
		Kuru	Tokabu	Other	Kuru	Tokabu	Other
A. Kuru	Adult	18	2	7	3	4	19
	Young ..	6	—	19	9	1	30
	Total	24	2	26	12	5	49
B. Tokabu	Adult ..	5	1	2	1	2	5
	Young ..	2	—	3	3	—	4
	Total	7	1	5	4	2	9
C. Other causes	Adult ..	45	11	39	—	13	73
	Young ..	3	2	9	1	3	24
	Total	48	13	48	1	16	97

four times the incidence in Group C (3/48). The fate of the male offspring in Groups A and C is strikingly different. Kuru killed 12 of the 66 sons of women dying of kuru, as compared with one only of the 114 sons in Group C.

The offspring of the small group of women who died of tokabu in Group B present a most interesting picture, since, although the numbers are very small, the situation appears to resemble Group A rather than Group C. Of the 15 sons recorded, 4 died of kuru. Two of these individuals were brothers, and a sister of theirs also died of kuru. Tokabu, as mentioned earlier, is a form of ritual murder performed on an individual believed to have induced kuru by sorcery. The resemblance found here between Groups A and B may well be coincidental, but it merits further investigation.

Of the 48 female deaths in Group C which were due to causes other than kuru or tokabu, 9 occurred at a young age. For males the corresponding figures are 24 out of 97. These proportions are similar to those found in the whole "R" file (Table I). In Group A, however, the situation is quite different; there is a very high incidence of deaths in childhood from other causes among the daughters (19 out of 26) and the sons (30 out of 49) of women who died of kuru. It might be thought that this could be due to the deaths of unweaned infants of mothers dying of kuru (since the Fore do not employ wet-nursing). However, analysis of the records suggests that this could account for at most only one half of this disproportionate mortality.

Analysis of the "K" and "Z" files has shown the same features to be clearly present.

A GENETICAL HYPOTHESIS

Study of this material suggested not only that a genetical basis was possible, but also that a simple model could be tentatively proposed.

Our suggestion is that kuru is controlled by a single autosomal gene "K", recessive to its allelomorph "k" in males, and dominant in females. In other words, males of the genotype KK are potential victims of kuru, whilst those who are Kk or kk are clinically normal.

TABLE V.

Genetical Hypothesis for Kuru on Basis of Single Autosomal Gene "K".

Genotype	Female	Male
KK	Kuru with early onset	Kuru with early onset
Kk	Kuru with late onset	Clinically normal
kk	Normal	Normal

Females of the genotype KK are potential victims at a young age, those of the genotype Kk are potential sufferers from the disease in adult life, and those who are kk are normal. This hypothesis is summarized in Table V.

If this theory is correct, then all males with kuru, being of the genotype KK, must have had mothers whose genotypes were either KK or Kk, and in either event these mothers would themselves be potential kuru victims. However, owing to the great variation in the age of onset of kuru, some of these mothers would die of other causes before kuru could be manifested.

It is not possible to say exactly what proportion of potential kuru victims is lost in

this way, but reference to Table I shows that of the 439 females who died of kuru, tokabu or other stated cause, 78 died before the age of 17 years and only 21 were kuru victims. Therefore about one-eighth of all females died in childhood from causes other than kuru. It may thus be argued that the risk of mothers with the genotype KK dying from causes other than kuru would be approximately one in eight, while mothers with the genotype Kk would have a considerably higher risk of death from other causes owing to the later onset of kuru in these individuals. Table VI gives the recorded causes of death of mothers of male victims of kuru in the three files "R", "K" and "Z". It may be seen that the situation is quite compatible with the genetical hypothesis. Five of the 28 mothers, about one-sixth, died from causes other than kuru (and it is of interest that three of these five maternal deaths were due to tokabu).

TABLE VI.

Deaths of Mothers of Male Kuru Victims[1]

File	Cause of Death		
	Kuru	Tokabu	Other
"R"	9	3	1
"K"	2	—	—
"Z"	12	—	1
Total ..	23	3	2

[1] Deaths of mothers (Kk or KK) of sons (KK) who died of kuru.

TABLE VII.

Deaths of Mothers of Young Female Victims of Kuru[1]

File	Cause of Death		
	Kuru	Tokabu	Other
"R"	6	3	2
"K"	—	—	—
"Z"	4	—	1
Total ..	10	3	3

[1] Deaths of mothers (Kk or KK) of daughters (KK) dying of kuru in early life.

Table VII illustrates in similar fashion the deaths of mothers (KK or Kk) of young females (KK) who were kuru victims. This group of young females may not, however, be all of the genotype KK, since there is presumably some overlap between the cases of early and late onset. Individuals included in this group on the basis of age of onset of kuru and who were actually genotype Kk could, of course, have derived the K gene from the father, and the mother need not have been a potential kuru victim. This factor, added to the general risk of death from other causes already mentioned, would lead one to expect a somewhat lower incidence of actual deaths from kuru among the mothers of this group, as compared with the previous group. It may be seen that the observations (10 out of 16 mothers dying of kuru) are in tolerably good agreement with the hypothesis.

Table VIII records the deaths of the mothers of adult female victims of kuru. The latter, presumed to be of the genotype Kk, could have received the gene K from either mother or father, and, in the absence of selection, at least one-half of the mothers of this group would be expected to carry the K gene and therefore be liable to develop kuru. Death from other causes prior to the onset of kuru would, as before, tend to reduce the proportion of deaths from kuru below this value. The actual observation is that 25 out of 77 mothers in this group died of kuru.

TABLE VIII.

Deaths of Mothers of Adult Female Victims of Kuru[1]

File	Cause of Death		
	Kuru	Tokabu	Other
"R"	16	5	34
"K"	—	—	1
"Z"	9	—	12
Total ..	25	5	47

[1] Deaths of mothers (KK, Kk, kk) of daughters (Kk) with late onset of kuru.

The family pedigrees contained in the "R", "K" and "Z" files, totalling some 250, have been examined in the light of this proposed genetical hypothesis, and it can be stated that no situation has been found throughout these files which is incompatible with the theory. The present survey failed to disclose clear examples of the junction of kuru and kuru-free lines. This was partly due to the fact that in the boundary areas, where some intermarriage with non-Fore occurs, this has been the custom for some considerable time, and it is not certain that the non-Fore people may not be carrying the gene in low frequency. This, together with the high incidence of kuru in the Fore area, prevents any firm conclusions being drawn from the pedigrees obtained. However, these

pedigrees contain many situations where prospective studies would provide a critical test of the present hypothesis.

DISCUSSION

The present study, together with the previous reports of Zigas and Gajdusek (1957) and Gajdusek and Zigas (1957, 1958), describes kuru as a disease widespread amongst the Fore, affecting males and young females almost equally around a mean age of about eight years in females and 14 years in males, and then attacking a larger proportion of adult females. around a mean age of about 33 years. We have shown that males affected by the condition are born almost exclusively to mothers with the disease, and that there is a marked tendency towards the same association for young female victims and their mothers.

The present study, which has led to a very tentative genetical hypothesis, must be regarded as essentially preliminary. A number of features have emerged which merit much closer study. The high death rate in childhood from causes other than kuru of the offspring of women dying of kuru (Table IV) requires explanation. The speculation arises as to whether some as yet unrecognized form of kuru or some condition related to it is occurring amongst these children. The precise relationship between deaths from kuru and those from "tokabu" needs to be explored. The genetical hypothesis proposed here will require further field study before it can be considered as established. On the basis of the theory, however, predictions can be made as to the likelihood that kuru will develop in a considerable number of individuals recorded in the pedigrees, and follow-up studies of these are clearly indicated. If males and young females with kuru are in fact homozygotes (KK), careful study of both parents would be of extreme importance in the search for some feature which might identify the presence of the K gene, other than by the development of the disease. Study of all available pathological material should be made to determine whether there are any recognizable differences between cases in males and young females on the one hand, and in adult females on the other.

The suggestion of a genetical basis for kuru should also be considered in the light of the negative results reported by Zigas and Gajdusek of their extremely extensive and thorough environmental studies. One of their articles (Gajdusek and Zigas, 1958) includes a number of examples of the development of kuru in circumstances which make it difficult to postulate a common environmental agent. Pathological studies (Gajdusek and Zigas, 1957; Fowler and Robertson, in the press) indicate a process of neuronal degeneration, without inflammatory reaction, which is compatible with either an abiotrophy or a toxic process. Clinically, the disease bears resemblance to some forms of spino-cerebellar degeneration (Simpson, Lander and Robson, 1958) which have been reported as familial disorders (Greenfield, 1954).

The establishment of a genetical basis for a condition with the features and frequency of kuru would be a matter of some difficulty under any circumstances. The present hypothesis is therefore advanced with much hesitation. The main justification for this presentation is the hope that it may assist in the direction of further investigations into this unique disease, which is both a challenge to modern medicine and a tragic problem for the Fore people.

ACKNOWLEDGEMENTS

The above report, and subsequent communications, were made possible only by the generous assistance and cooperation of the University of Adelaide and of the Department of Territories of the Commonwealth. We are indebted to many members of the Administration of Papua and New Guinea, particularly Dr. R. F. R. Scragg, Acting Director of Health, and Mr. J. Baker, Patrol Officer, Okapa, for invaluable assistance. Grateful acknowledgement is due to Dr V. Zigas and Dr. C. Gajdusek, who freely made their unique information and experiences available to us.

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CRYPTOCOCCOSIS (TORULOSIS): A REPORT OF NINE CASES[1]

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SUMMARY

Five cases of cryptococcosis occurred in South Australia during 1957. Only four other cases (also described here) were recognized in the previous ten years. In four cases in this series the patient had a preceding respiratory infection followed by pulmonary cryptococcosis, and three of these developed central nervous system involvement. Isolated pulmonary lesions, cerebral granuloma and spinal cord involvement are rare forms of cryptococcosis which also occurred in this series.

No treatment has proved effective; "Amphotericin B" and "Fungizone", now under trial, were unsuccessful in two cases here. Surgical removal of isolated brain and lung lesions may help, but earlier diagnosis, perhaps by the development of immunological tests, is highly desirable. Because of its varied manifestations, cryptococcal meningitis is difficult to diagnose until late in the course of the disease.

The cause of human infection is unknown, but it must be related in some way to the resistance of the host, as well as to variations in virulence or type of the fungus. The significance of contact with pigeons is discussed.

CRYPTOCOCCOSIS, perhaps better known as torulosis, is a mycotic infection caused by *Cryptococcus neoformans*. On culture the yeast-like cell usually varies from four to seven microns in diameter. The cell is surrounded by a mucinous capsule, which may vary in thickness from seven to eighteen microns (Wicker and Warren, 1948).

Cryptococcus neoformans is one of seven species of the genus *Cryptococcus*. There are pathogenic and non-pathogenic varieties. The pathogenic strains grow on culture media at 37° C. and are virulent when injected into mice. They have been isolated from peaches, milk and soil and recently from pigeons' manure (Emmons, 1955). They have also been recovered from the skin, intestine and pharynx of humans, where they lead a saprophytic existence. Morphologically non-pathogenic strains of *Cryptococcus neoformans* are similar to the pathogenic varieties; however, they are less virulent towards mice (Littman and Zimmerman, 1956). Avirulent strains of *Cryptococcus neoformans* are widespread in animal and vegetable matter (Benham, 1935).

Once considered a rare disease, cryptococcosis is now becoming recognized with greater frequency, especially in the United States, where over three hundred cases have been reported. In Australia the disease has been recorded on relatively few occasions.

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The first known instance of cryptococcosis in Australia occurred in 1915; it was reported by Professor J. B. Cleland in 1927. In this case, the clinical manifestations were limited to a large mass in the right iliac fossa, which was thought to be an inoperable sarcoma. The mass was removed and was shown to contain torula cells. Attempts at culture, however, were unsuccessful, and inoculated guinea pigs and rabbits remained healthy for one year. No autopsy was performed upon the man when he died a year later. This may have been a rare example of localized cryptococcosis occurring outside the lungs.

The first case of cryptococcal infection of the central nervous system diagnosed in Australia during the patient's life was reported by Swift and Bull, also in Adelaide, in 1917. Torula cells were found in the cerebro-spinal fluid, and the organism was probably *Cryptococcus neoformans*, although there was an attempt at what appeared to be mycelial formation on cultures. These were probably chains of budding cells.

Other Australians who have contributed to the literature of cryptococcosis in recent years are C. T. Champion de Crespigny (1944), Piper (1944), Cox and Tolhurst (a valuable monograph) (1946), Alderman (1949), Starr and Geddes (1949), McConchie (1951), Susman (1954), Buckle and Curtis (1955), Fortune *et alii* (1955) and Geaney *et alii* (1956).

Nine cases of cryptococcosis are presented in this series, in eight of which the subjects have

TABLE I.

Summary of Findings in Nine Cases of Cryptococcosis

Case No.	Occupation	Age (Years)	Presenting History	Clinical Diagnosis	Duration of Disease	Treatment	Remarks
1	Miner	35	Headaches for three weeks	Subdural hæmatoma	6 weeks	Penicillin	History of head injury. Diagnosis at autopsy
2	Home duties	45	Headache for two weeks	Meningitis	6 weeks	Penicillin	Quiescent pulmonary focus
3	Farmer	27	Headache and cough followed by right basal pleurisy	Unresolved pneumonia	——	Intrapleural "Actidione"	Pulmonary lesion excised. Well 18 months later
4	Window dresser	22	Headache and vomiting for three weeks	Tuberculous meningitis	5 weeks	Intramuscular and intrathecal "Actidione"	Opacity in chest X-ray film
5	Nursing sister	35	Pleuritic pain and meningeal irritation for five weeks	Meningitis	4 months	Intraventricular and systemic "Actidione". Penicillin. Streptomycin	Lung lesion clinically. Lobectomy performed. *Cryptococcus neoformans* isolated from sputum and cerebro-spinal fluid
6	Home duties	72	Stiff neck and headache for one month. Became comatose after fluctuating drowsiness	Subdural hæmatoma	5 weeks	Systemic "Actidione"	No lung lesion clinically or at autopsy
7	Commercial agent	55	Sudden onset of giddiness, together with signs in left arm and leg	Cerebral tumour	11 weeks	Penicillin	Diagnosis at operation
8	Process worker	34	Headache and weakness for 11 weeks	Encephalitis	3 months	"Amphotericin B" and "Fungizone"	Contact with pigeons. Cranial nerve palsies. Spinal cord symptoms
9	Clerical duties	51	Headaches for 12 months. Epilepsy for eight months	Cerebral tumour	14 months	"Fungizone"	Resection of cerebral granuloma

come to autopsy during the past eleven years. Five have occurred in South Australia during 1957. One patient is still living. Table I lists some relevant facts about each patient.

PULMONARY LESIONS

It has been suggested that *Cryptococcus neoformans* may gain entry into the body through a number of sites, viz. pharynx, tonsils, nose, skin and gastro-intestinal tract; however, the respiratory tract is considered the most usual portal of entry (Terplan, 1948). Many patients give a history of a preceding respiratory infection. In this series four patients developed cryptococcal lesions of the lungs after a respiratory tract infection.

Table II shows the organs that were involved in each of the nine cases. The lungs were involved in five cases, the general distribution of lesions conforming to those described by Haugen and Baker (1954) as follows: (i) Confined to small subpleural nodules less than 1·5 centimetres in diameter, without other evidence of fungal infection. (ii) As in (i), but with lesions elsewhere (Case 2). (iii) Larger pulmonary lesions with or without central nervous system involvement (Cases 1, 3, 4 and 5). (iv) Miliary pulmonary lesions with foci elsewhere. (v) No involvement of the lungs (Cases 6, 7, 8 and 9).

Cerebral and Pulmonary Lesions

The first patient is an example of cryptococcal meningitis following a respiratory tract infection:

CASE 1.—A man, aged 35 years, was a miner at Broken Hill. Six weeks before death he had a severe cold with fever, lassitude and loss of weight. After this he developed a constant headache, which he attributed to a fall from a bicycle several weeks before. He was admitted to hospital because of severe headache, diplopia and generalized weakness and was transferred to the Royal Adelaide Hospital ten days later, on July 5, 1946.

Examination showed him to be restless and irritable, with slurred speech, complete flaccid paralysis and bilateral papilloedema. An air ventriculogram was normal. The cerebro-spinal fluid was clear and colourless with a pressure of over 400 millimetres of water, and there were 500 leucocytes (20% polymorphs, 80% lymphocytes) per cubic millimetre. The protein, chloride and sugar levels were normal, and there was a slight excess of globulin. A direct smear showed a few Gram-positive bacilli, and on culture a scanty growth of a non-hæmolytic staphylococcus and a diphtheroid were obtained. Serial cerebro-spinal fluid examinations showed high lymphocyte counts and a protein level of 90 milligrammes per 100 millilitres.

Torula cells were not seen. The results of a Wassermann test and chest X-ray examination were both negative. Despite parenteral treatment with penicillin he died fourteen days after admission to hospital.

Autopsy revealed an infarct-like area measuring seven by seven centimetres with a well-defined border at the base of the left lung. The surface of the brain appeared congested, but there was no clouding of the membranes. Examination of the ventricular fluid revealed torula cells, and histological examination of sections from the lung and meninges confirmed that the lesions were due to *Cryptococcus neoformans*.

In four patients (Cases 1, 3, 4 and 5) the illness was preceded by a respiratory tract infection, and each of these had cryptococcosis of the lungs

TABLE II.

The Organs Involved by Cryptococcus Neoformans

Case Number	Lungs	Brain	Meninges
1	+ (Left lower lobe. Inflammatory reaction)	—	+
2	+ (Right hilar region. Quiescent focus)	No record	+
3	+ (Right lower lobe. Inflammatory reaction)	—	—
4	+ (Right upper lobe. Mucoid lesion)	+ (Basal ganglia)	+
5	+ (Right lower lobe. Inflammatory reaction)	+ (Cerebrum and cerebellum)	+
6	—	—	+
7	—	—	+
8	—	+ (Cerebellum)	+ (Spinal cord involvement)
9	—	+ (Toruloma of brain)	+

detected either clinically or at autopsy, as shown in Case 1. In the other five cases, in which there was no preceding respiratory illness, no lung lesions were found clinically or at autopsy.

Quiescent Lung Focus

The occurrence of small isolated lesions in the lungs of some patients with cryptococcal meningitis, or in those dying of unrelated diseases, supports the theory that the respiratory tract is the site of entry of the cryptococcus. Baker and Haugen (1955) stress the fact that foci of cryptococcosis in the lungs may be very small indeed, and may be found only by careful searching. The smaller foci are often granulomatous, with a good deal of surrounding fibrous tissue, and are probably quiescent. In this they resemble the behaviour of tubercles, and like tubercles they may apparently become active again.

In the following case of cryptococcal meningitis a small quiescent focus in the lung may have given rise to a terminal meningeal infection. Exogenous reinfection cannot be excluded.

FIGURE I

Quiescent focus of pulmonary cryptococcosis with torula organisms in the central cavity (Case II). (Hæmatoxylin and eosin. ×30)

CASE 2.—A woman, aged 45 years, was admitted to the Royal Adelaide Hospital on March 6, 1949, with a history of long-standing anæmia complicated by severe nausea, vomiting and fever in the week before admission. She died after several weeks, having developed cryptococcal meningitis, which was proved by growing a positive culture from the cerebro-spinal fluid.

At autopsy the right lung contained a small nodule two centimetres in diameter near the hilum. This had a greyish appearance with a well-defined edge. Microscopic examination of sections showed this to be a focus of cryptococcosis with a central cavity containing torula cells surrounded by a thick fibrous capsule (Figure I). The surface of the brain showed no abnormality, but histological examination of sections of the meninges revealed torula cells, with a minimal inflammatory reaction.

Resectable Pulmonary Lesions

Isolated Pulmonary Lesion.—Involvement of the lungs alone is shown in the following case.

CASE 3.—A man, aged 27 years, had been healthy until mid-June, 1956. At that time he developed a retro-orbital headache, but continued working for one week. He then developed acute malaise and pain in the eyes, and pyrexia of 102°F. Apart from a slight unproductive cough he had no other symptoms and was put to bed for one week. During this time he remained feverish.

On July 12, 1956, he suddenly developed right basal pleurisy and was treated with penicillin for one week. After two days of this treatment he began to improve, but the signs of impaired percussion note and diminished breath sounds persisted at the right lung base.

Investigations at this time showed 16,000 white cells per cubic millimetre, with polymorphs 64%, lymphocytes 21%, monocytes 11% and eosinophils 4%. Agglutination tests and Mantoux tests (1 in 1000) gave negative results. An X-ray examination of the chest taken on July 14 showed an opacity at the right lung base.

On July 20, he felt well though somewhat weak. There was very little cough and no sputum. A further X-ray examination of the chest confirmed the presence of an opacity suggestive of hydatid cyst. The white blood count remained elevated at 16,800 cells per cubic millimetre, with polymorphs 69%, lymphocytes 18%, monocytes 8% and eosinophils 5%. The Casoni test gave a weak immediate positive reaction and a negative delayed reaction.

Thoracotomy was carried out, and a large granulomatous tumour " the size of a billiard ball " was found in the lower lobe of the right lung. This was recognized as cryptococcosis, and the posterior segments of the right lower lobe containing the mass were excised. Post-operatively a small effusion developed at the right lung base, and the aspirated fluid was found to contain torula cells. " Actidione " was injected into the pleural cavity on three occasions. After this, torula cells were no longer found in the pleural fluid. He has remained well since discharge from hospital eighteen months ago.

Pulmonary cryptococcosis is unusual without central nervous system involvement, and it may be that this case is an example of cryptococcosis localized to the lungs.

Surgical resection of involved lung tissue was first advocated by Taber as long ago as 1937. Since then there have been several reports of lung lesions that have been removed without associated evidence of meningeal involvement (Dormer *et alii*, 1945; Froio and Bailey, 1949; Starr and Geddes, 1949; McConchie, 1951; Berk and Gerstl, 1952; Baker, 1952; Cruikshank and Harrison, 1952; Corpe and Parr, 1954; Susman, 1954; Poppe, 1954; Nichols and Martin, 1955; Pinney, 1956; Webb and Biggs, 1956). Varying lengths of remission have been claimed, the longest being eight years in the case originally recorded by Froio and Bailey. Prolonged follow-up is essential, however, as a patient reported by Palmrose and Losli (1952) relapsed four years after surgical resection. It would seem that pulmonary cryptococcosis may produce a symptomless nodular lesion similar to other granulomatous diseases and therefore should enter into the differential diagnosis of circumscribed lesions in the lungs.

Lung Lesion in Combination with Meningitis.—In two cases of the series the patients had demonstrable lung lesions in combination with cryptococcal meningitis.

CASE 4.—A male, aged 22 years, noticed that two weeks before admission to hospital his head ached on bending over. This gradually became more severe, and he began to vomit. There was a history of a cold six months previously with excessive morning sputum since.

He was admitted to the Royal Adelaide Hospital on December 15, 1955. On examination he looked pale and toxic, and there was moderate neck stiffness. The only other positive finding was venous engorgement of the fundi oculorum. Lumbar puncture at this stage showed the fluid to be clear and colourless, with 138 lymphocytes and 145 polymorphs per cubic millimetre. The fluid contained 80 milligrammes of protein, 27 milligrammes of sugar, and 730 milligrammes of chloride per 100 millilitres. There was a definite excess of globulin. Direct smear examination and culture failed to reveal *Cryptococcus neoformans*. A chest X-ray examination revealed a rounded opacity adjacent to and above the right hilum, which was thought to be due to enlarged glands.

Subsequently torula cells were isolated from the cerebro-spinal fluid, and the culture became positive. The patient slowly developed signs of increased intracranial pressure and died on December 27, 1955. This patient was treated with intramuscular and intrathecal injections of " Actidione ", an antibiotic isolated from *Streptomyces griseus* (10 milligrammes given intrathecally and 20 milligrammes intramuscularly every six hours).

At autopsy the right lung contained a mass five by four by five centimetres in the hilar region of the upper lobe (Figure II). It was well demarcated from the surrounding compressed lung, and its substance showed loose, pale, branching trabeculæ widely separated by clear mucinous material. The brain was " slippery " on removal, with an excess of slightly opaque fluid between the pia and arachnoid (rather characteristic features of cryptococcal meningitis). A few small gelatinous nodules were seen over the surface. Horizontal section of the brain through the cerebral hemispheres showed a large number of very small clear " white-anted " areas in the basal ganglia, particularly on the left side.

Microscopically torula cells were seen in the lung, brain and meninges. There was only slight meningeal inflammatory reaction (Figure IV).

The treatment with " Actidione " in this case was disappointing, for the patient showed no signs of recovery and there was no decrease in the torula cell count in the cerebro-spinal fluid. We may note that after reviewing the known cases of cryptococcal meningitis treated with " Actidione ", Carton (1952) concluded that this drug was not very effective against central nervous system cryptococcosis.

When torula organisms invade the body the cellular reaction is composed mainly of lymphocytes, giant cells and macrophages.

Polymorphonuclear leucocytic reaction, necrosis and tubercle formation occur only in a minority of patients (Baker and Haugen, 1955). (See Table III.)

TABLE III.

Microscopic Features of Lesions in Twenty-Six Cases of Torula Infection (Baker and Haugen, 1955)

Feature	Number
Polymorphonuclear leucocytic reaction	5
Lymphocytic response	22
Macrophage response	21
Giant cell response	23
Caseous necrosis	4
Fibrosis	15
"Tubercle" formation	2
Calcification	0

Lesions in the lung itself fall into two main groups: (i) massive accumulations of cryptococci and a lack of inflammatory response as in the case just described (Figure II); (ii) few

FIGURE II

Localized pulmonary lesion showing peripheral cavitation and central trabeculation (Case IV). (×2)

organisms and a greater inflammatory reaction, the masses being firm and rubbery as seen in Case 5 (Figure III).

Some recent experimental work (Levine *et alii*, 1947) has shown rather an interesting correlation between the lack of inflammatory response and the predilection of the condition for the central nervous system. Certainly in the brain and meninges of the patient in Case 4 and the others with cerebral involvement, the cellular response was far less than that shown in other organs. In Case 4 the patient also had a marked cystic lesion of the lung with minimal cellular response,

FIGURE III

Pulmonary "toruloma" with sharply defined outline (Case V). (×1·5)

and in addition he had pronounced cerebral lesions, denoting a general lack of inflammatory response. As pointed out by Littman and Zimmerman (1956), the lung lesions in cryptococcosis expand by compression of surrounding tissue rather than by destruction of it. This was well shown in the histological sections from this patient, there being normal, compressed lung adjacent to the relatively acellular cystic lesion.

CASE 5.—A female, aged 35 years, had a severe cold and sore throat five weeks before admission to the Royal Adelaide Hospital. She then developed pleuritic pain in the right axilla. One week later she developed continuous severe headache, which was most severe in the occipital region and behind the eyes. She was admitted to Northfield Infectious Diseases Hospital,

where the diagnosis of cryptococcosis was made after a lumbar puncture, and she was transferred to the Royal Adelaide Hospital on January 22, 1957.

Examination revealed diminished air entry at the right lung base and slight neck stiffness. A chest X-ray examination showed an opacity at the right lung base, and on January 24 a right lower lobe lobectomy was performed. The right lower lobe contained a circular white mass eight centimetres in diameter, which presented on the diaphragmatic and costal surfaces of the lung (Figure III). It appeared to have infiltrated the wall of the main bronchus which led away from the mass. Microscopic study of the "toruloma" revealed a granulomatous lesion with relatively fewer organisms and a much denser meshwork of associated fibrous tissue than in the previous case, and it was considered that this lesion was in the process of healing.

Subsequently the patient developed central nervous system involvement with signs of increased intracranial pressure. She was treated initially with systemic administration of "Actidione", 125 milligrammes twice a day. The torula cell count in the cerebro-spinal fluid then rose from two to 52 cells per cubic millimetre in ten days, and she developed increasing headache and looked very ill. An external ventriculostomy was performed on March 13 to enable intrathecal "Actidione" therapy to be used and to control the cerebro-spinal fluid pressure. Twenty milligrammes of "Actidione" were given twice a day together with penicillin and streptomycin. The concentration of "Actidione" in the cerebro-spinal fluid was maintained at two microgrammes per cubic millimetre, which was considered an adequate therapeutic level (Carton, 1952). After thirteen days the ventriculostomy was closed, as the patient showed no signs of recovery and the torula cell count was rising. Her mental state slowly deteriorated, and she died on April 20, 1957.

At autopsy the brain was extremely slippery to handle, the leptomeninges were clouded, and there was a general slight nodularity of the brain surface. Section of the brain showed several small colonies of the organisms, the largest, in the head of the right caudate nucleus, measuring five by two by five millimetres. There were others in the grey matter of the frontal and parietal lobes which were very much smaller, mostly less than one millimetre in diameter, with a slightly larger colony in the right dentate nucleus of the cerebellum.

This patient had a positive sputum culture for *Cryptococcus neoformans* prior to operation, and it is interesting that the lesion could be seen penetrating a main bronchus.

INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM

Cryptococcal Meningitis

CASE 6.—A female, aged 72 years, was in good health and led an active life until one month before admission to the Royal Adelaide Hospital. She developed a stiff neck after picking fruit and two days later noticed occipital and frontal headache, which gradually became worse. She was then admitted to a country hospital, where she stayed for a fortnight. During this time she had episodes of drowsiness and mental changes. On the night before admission to the Royal Adelaide Hospital she became comatose.

Examination on June 5, 1957, revealed crepitations at both lung bases. The cardio-vascular system was normal, and neurological examination showed absence of limb reflexes. There were, however, no focal neurological signs. A lumbar puncture was performed in the country hospital and revealed a pressure of 360 millimetres of water, the fluid being clear and colourless. X-ray examination showed no abnormality of the skull or of the chest apart from an enlarged left ventricle.

A diagnosis of subdural hæmatoma was made, even in the absence of a history of head injury, but exploratory burr holes failed to confirm this.

Next day the cerebro-spinal fluid contained 16 torula cells per cubic millimetre. Treatment with intramuscular injections of "Actidione", 250 milligrammes twice a day, was begun, but the patient died on June 10, 1957.

At autopsy a careful search of the lungs, both macroscopic and microscopic, revealed no evidence of cryptococcosis. Although the brain showed no abnormality macroscopically, there was marked involvement of the meninges microscopically.

It was noticed that this patient's brain and the brains from the patients in Cases 5 and 8 appeared normal macroscopically in the autopsy room. However, after fixing in 10% formol saline for fourteen days, the small white lesions of cryptococcal meningitis became clearly visible.

This patient lived for only five weeks after the onset of the illness, apparently having (like the patient in Case 4) a very poor defensive response to the cryptococcus, as shown by the minimal inflammatory reaction in the meninges. As Baker and Haugen (1955) point out, the cellular response is mainly by lymphocytes and polymorphs in the early stages of the disease, and it is not until six or eight weeks have elapsed that giant cells and a chronic inflammatory reaction become evident.

The following case also illustrates involvement of the meninges without pulmonary lesions.

CASE 7.—A man, aged 55 years, was admitted to hospital at Springbank on April 19, 1949. He had been quite well until six weeks previously, when he suddenly became giddy and fell over while playing bowls. After this incident he developed, in succession, increasing unsteadiness on standing or walking, with a tendency to fall to the left; headache, both frontal and occipital, which was not severe; uncontrollable twitching of the left side of the body, mainly in the hand; and paræsthesia over the left side of the face.

The patient gave a clear history, and during the examination there were frequent involuntary jerkings of the left arm, left side of the abdomen and left leg. General physical examination revealed no abnormality. The neurological examination showed that the cranial nerves were intact, except for moderate perception deafness, and all forms of sensation were normal. The superficial reflexes were normal, except for the abdominals, which were absent on the right side. There was gross ataxia of the left arm and left leg.

Ventriculography was performed on April 27. The brain bulged into the burr hole to a moderate degree, suggesting raised intracranial pressure, but subsequent X-ray examination showed no hydrocephalus or displacement of the ventricular system. The ventricular cerebro-spinal fluid contained five to ten red corpuscles per high power field but no leucocytes. The

protein and chloride contents were normal. Lumbar puncture two days later revealed a normal fluid under normal pressure. A chest X-ray film was normal.

During the next fortnight the patient's condition deteriorated. His speech became slurred, and he was quite drowsy. Left-sided papillœdema developed soon afterwards.

On May 19, a diagnostic craniotomy was performed because the bulk of the evidence suggested a cerebral tumour. At operation the leptomeninges were infiltrated with small white soft granular foci, which were distributed mainly along the course of veins. The pia-arachnoid had a diffuse ground-glass appearance.

A direct smear examination of sections taken revealed torula-like cells. The patient died on May 26 after an illness lasting eleven weeks.

Autopsy revealed cryptococcal involvement of the leptomeninges with no intracerebral lesions or involvement of the lungs. Microscopically the meninges showed many torula cells with only a mild inflammatory exudate.

Cryptococcal meningitis may present in many obscure forms. Carton and Mount (1951) in a study of 178 cases of cryptococcal infection of the central nervous system, found that surgical procedures had been carried out in one quarter of the cases, because of evidence of an expanding intracranial lesion. Cases 1 and 7 show the diversity of presentation of cryptococcal meningitis.

Spinal Cord Involvement

Involvement of the spinal cord by the cryptococcus is rare. Carton and Mount (1951) were able to find only ten cases of cryptococcal lesions of the spinal cord in the literature. In only three of these did the patient show spinal cord symptoms, the remainder having incidental lesions at autopsy. Since then I have found only two more cases of granulomata of the spinal cord, in both of which the patient presented with spinal cord symptoms and had the granulomata successfully resected (Ley *et alii*, 1951; Ramamurthi and Anguli, 1954).

CASE 8.—A man, aged 34 years, was quite well until June, 1957, when he developed a severe generalized headache. He was referred to the Out-Patients Department of the Royal Adelaide Hospital, where examination revealed no gross abnormality. As the headache persisted, he was admitted to hospital for investigation on July 5, 1957. General examination again showed no abnormality, but neurological examination revealed absence of the right knee jerk and some loss of sensation in the right lower leg.

Skull and chest X-ray examinations and left carotid angiography showed no abnormality. There was a leucocytosis of 15,300 cells per cubic millimetre. The serial cerebro-spinal fluid examinations are set out in Table IV. The patient was discharged from hospital on July 20.

On August 7 he was readmitted with a history of increasing weakness and continued severe headache. On examination he was sleepy and semi-cooperative. The fundi showed slight venous engorgement. Again the only positive neurological sign was absence of the right knee reflex and doubtful sensory loss. The patient disclosed during this admission that he had been closely associated with pigeons several years previously.

Two days after admission the headache became more severe, and he complained of neck stiffness. Torula cells were now found in the cerebro-spinal fluid, and *Cryptococcus neoformans* was cultured. Slowly his general condition and vision deteriorated, and he developed bilateral sixth nerve palsy.

On August 16 treatment with intravenous injections of "Amphotericin B" was begun, the dosage being 50 milligrammes in the first six hours and thereafter 50 milligrammes daily. With this treatment his condition improved, he became cooperative, and his headache became less severe. On August 28 supplies of "Amphotericin B" were exhausted. Subsequently he relapsed and developed bilateral papillœdema together with a left facial palsy. Intravenous administration of "Fungizone" was begun on September 10, the dosage being similar to that of "Amphotericin B"; however, the patient died suddenly on September 21, 1957.

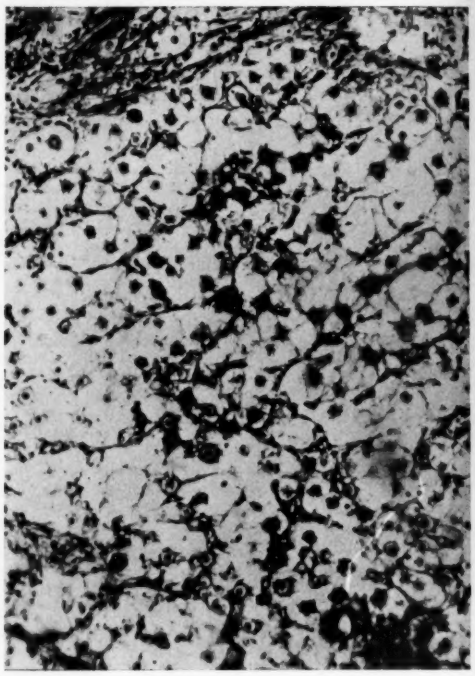

FIGURE IV

Meningeal exudate showing many torula cells but few inflammatory cells (Case IV). (Hæmatoxylin and eosin. ×40)

At autopsy no focus of cryptococcosis could be found in the lungs. The brain had the characteristic slimy feel, and there were numerous small, circular, low elevations on its surface, particularly on the inferior aspects of the frontal lobes. Horizontal section showed no abnormality, but a single gelatinous lesion was present in the left dentate nucleus of the

cerebellum. The spinal cord exhibited many small raised patches of cryptococcosis in the meninges up to 0·5 centimetre in diameter, especially in the lumbar region.

Microscopically the lesions showed the characteristic picture of cryptococcosis without much inflammatory exudate. However, there were several foci in the lumbar region, which showed an intense granulomatous

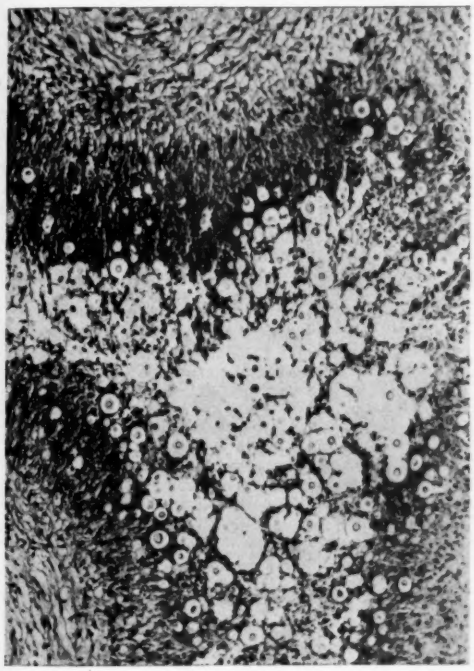

FIGURE V

Granulomatous lesion in the meninges of the spinal cord showing torula cells surrounded by necrotic debris and palisaded cells (Case VIII). (Hæmatoxylin and eosin. ×35)

reaction (Figure V) and intimal proliferation of small blood vessels. These were apparently older lesions than elsewhere in the meninges. Several of the posterior lumbar nerve roots showed a central fibre degeneration (Figure VI), which appeared to be continuous with similar early lesions in the posterior horns of the cord.

It would appear that these granulomatous lesions were responsible for the patient's initial spinal cord symptoms. The history of close contact with pigeons in this case is interesting, because Emmons (1955) was able to isolate pathogenic strains of *Cryptococcus neoformans* from pigeons' nests and droppings. He pointed out that their mode of nesting probably made a suitable site for the growth of the yeast, for the droppings remain in the nest.

The test of pathogenicity used by Emmons was to inject a saline suspension of the specimens intraperitoneally into mice. The mice were then killed three to four weeks later and examined for evidence of cryptococcosis. However, it has not been proved that the strains pathogenic to mice are capable of infecting humans, for the criteria of pathogenicity in man are still unknown. On the other hand, human pathogenic strains will infect mice.

The cerebro-spinal fluid findings in Case 8 are recorded in detail, for this was the first case in which the patient was treated with "Amphotericin B" and "Fungizone" in South Australia. These figures show how invariably the fluid was under increased pressure. In all

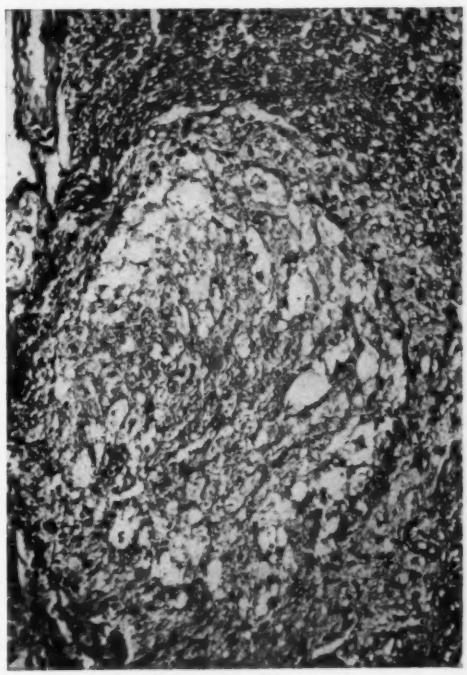

FIGURE VI

Fibre degeneration of a posterior nerve root (Case VIII). (Hæmatoxylin and eosin. ×40)

cases the predominant cell was the lymphocyte, though some did show quite a high level of polymorphonuclear leucocytes. This was so in Case 4, although the inflammatory reaction was minimal and the cerebral lesions were severe. In Case 8 the patient at no stage showed high levels of leucocytes, and yet the meningeal reaction was quite marked. This indicates that

TABLE IV.

Results of Cerebro-Spinal Fluid Examinations in Case 8 during Treatment[1]

Date	Pressure (Millimetres of Water)	Appearance	Cells per Cubic Millimetre			Globulin	Milligrammes per 100 Millilitres			Smear (Cells per Cubic Millimetre)	Culture for *Cryptococcus neoformans*
			Red Blood Cells	Lympho-cytes	Poly-morphs		Protein	Chloride	Sugar		
First admission:											
16.7.57 ..	270	Turbid	365	275	30	Excess	75	714	69	Negative	Negative
Second admission:											
9.8.57 ..	400+	Clear	—	90	25	—	50	—	—	35	Positive
16.8.57 ..	400+	Turbid	—	60	28	—	35	662	94	300	Positive
19.8.57 ..	400+	Turbid	770	52	4	Excess	40	673	61	88	Positive
23.8.57 ..	400+	Clear	50	5	10	Excess	45	659	60	70	Positive
28.8.57 ..	400+	Clear	38	11	4	Excess	40	—	48	28	Positive
9.9.57 ..	400+	Turbid	10	38	10	—	105	697	58	10	Positive
12.9.57 ..	500+	Clear	10	44	14	Excess	125	658	48	4	Positive
19.9.57 ..	500+	Turbid	—	—	—	—	—	—	—	—	—
21.9.57 ..	Death	—	—	—	—	—	—	—	—	—	—

[1] Treatment consisted of intravenous administration of " Amphotericin B ", August 16 to 28, and intravenous administration of " Fungizone ", September 10 to 21.

high levels of lymphocytes in the cerebro-spinal fluid may accompany a chronic inflammatory and granulomatous reaction in the meninges.

The protein levels varied greatly. The rise in Case 8 was in accordance with high levels previously recorded in other similar cases. Case 4 was the only one to show a significant fall in the levels of chloride and sugar, which have been described previously and may suggest a diagnosis of tuberculous meningitis.

The torula cell count appeared unaltered by treatment except in Case 8. Here, with the aid of " Amphotericin B " and " Fungizone ", it fell from 300 cells to 10 cells per cubic millimetre. This was associated with quite a marked improvement in the patient's condition.

Cerebral Granuloma

Granulomatous lesions due to the cryptococcus in the brain are unusual. Carton and Mount (1951) recorded nine cases, Balkrishna and Lilauwala (1952) one case, Liu (1953) six cases, Manganiello and Nichols (1955) one case. In the following case the patient was thought to have a parasagittal meningioma prior to operative removal.

CASE 9.—A woman, aged 51 years, had been quite well until twelve months previously, when she developed headaches over the vertex. At first these occurred only on movement and lasted a few seconds. Over a period of months the headaches became more frequent, but still lasted less than two minutes and were relieved by rest.

Eight months before admission to hospital she began to have epileptic seizures, commencing with a feeling of fear and claustrophobia. For the two months prior to her admission to hospital she had short episodes of vertigo and ataxia. She was admitted to the Royal Adelaide Hospital on November 24, 1957.

Examination revealed that she was an alert and sensible woman. The fundi showed bilateral early papilloedema, the left more than the right, and there was a small hæmorrhage at the margin of the left disc. The findings of general physical examination were normal, but the neurological examination revealed a mild left hemiparesis involving the face, arm and leg. Soon after her admission she had several seizures resembling both *petit* and *grand mal* fits.

A skull X-ray examination showed prominent diploic channels in the right parietal region. Right carotid arteriography and ventriculography both confirmed the presence of a space-occupying lesion of the superior parietal region near the midline. After the ventriculography a craniotomy was performed on December 11. The dura was opened close to the sagittal sinus, and just beneath the cortex in the midline a faintly lobulated tumour was seen. It was avascular and easily enucleated. The tumour was five centimetres in diameter and was composed of several small lobules containing gelatinous material. Several of these ruptured during removal, and the contents fell into the wound.

Microscopically the lesion consisted largely of encapsulated torula cells. There was a marked cellular reaction composed largely of lymphocytes and, most unusually, plasma cells.

Post-operatively the patient's condition was satisfactory, but after ten days she became drowsy and unaware of her surroundings. On January 2, 1958, intravenous administration of " Fungizone " was commenced (0·25 milligramme per kilogram of body weight on the first day, increased to 1·0 milligramme per kilogram of body weight per day, by increases of 0·05 milligramme per kilogram of body weight per day). After several days, intravenous administration of heparin was combined with the " Fungizone " to counteract thrombophlebitis.

The results of cerebro-spinal fluid examinations of this patient were all negative for torula cells until January 6, 1958, when a positive smear and culture were obtained. The patient died suddenly on January 25, the salient clinical feature being marked hypotension, similar to the terminal stages in Case 8.

Clinically and at autopsy there was no evidence of pulmonary cryptococcosis. This emphasizes the fact that a localized cerebral granuloma may be the only evidence of systemic cryptococcal infection.

These last two cases illustrate the problem of diagnosis in cryptococcal meningitis. As yet the isolation of torula cells from the cerebro-

spinal fluid by India ink preparations is the only certain method of diagnosis. In the case just described the patient had been unwell for nearly twelve months before the diagnosis was made, and Case 8 had a history of nearly three months before torula cells were isolated from the cerebro-spinal fluid.

Some method must therefore be found to enable an earlier diagnosis to be made before such drugs as " Amphotericin B " and " Fungizone " can be given a satisfactory trial. At the moment they are being used when probably the disease is too far advanced. It is hoped that further research into precipitin and complement-fixation reactions, together with skin hypersensitivity tests, may significantly assist earlier diagnosis.

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THE EFFECT OF TIGLOIDINE ON EXTRAPYRAMIDAL SYNDROMES (HUNTINGTON'S CHOREA)[1]

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SUMMARY

The tiglic esters of tropine and pseudo-tropine (tigloidine) were tried in 12 cases of Huntington's chorea. Temporary, occasionally dramatic improvement was obtained in some cases and confirmed by withdrawal of the drug, the use of placebos and renewal of the original medication.

The effective dose of the drug varied from case to case and ranged from 100 to 400 milligrammes per day. The minimal effective dose was found to be very near to the optimal dose; no further improvement could be obtained by further increasing the dosage.

No unpleasant side effects were observed in the subjects treated. No habituation was observed.

In no case could all the symptoms of the illness be affected. In some cases mainly the motor disturbances were improved; in others there was mainly an improvement in mood, the patients becoming sometimes distinctly euphoric. The two effects did not seem to be connected, nor was the action of the drug predictable.

The results are discussed. The investigation is being extended with the purpose of studying the dual action of the drug. Promising results have so far been observed in cases of pure depression as well as in pure motor-disturbances (post-traumatic spinal spastic paraplegias). These results are being prepared for publication.

DURING a pharmacological investigation of the minor alkaloids occurring together with hyoscyamine and hyoscine in the plants *Duboisia myoporoides* and *D. Leichhardtii* (Solanaceæ) it was found that the by-alkaloids containing tropine or tropine homologues esterified with aliphatic acids showed very little toxicity and lacked any clear-cut pharmacological action, with the single exception of tiglyl-ψ tropine (tigloidine). This alkaloid likewise was of very low toxicity and lacked the action of atropine on eye, sweat, salivary and intestinal glands, and on heart and smooth intestinal muscle. It exhibited, however, distinct physiological activity. In dogs rendered overexcitable by "Methedrine" (one milligramme per kilogramme), the intravenous injection of 10 milligrammes per kilogramme of tigloidine abolished within a few minutes the restlessness and motor overactivity. In the isolated sartorius muscle of the toad *Bufo Marinus*, tigloidine showed in almost equal concentrations the same depressant effect on excitation, propagation and recovery after single or repetitive electrical stimulation as atropine (Trautner and McCallum, 1950). On these findings it was decided to try the alkaloid in clinical conditions in which excessive motor activity is a prominent symptom. Beneficial effects were observed in some cases of Parkinsonism (Trautner and Noack, 1951). The present publication records observations made in a series of cases of Huntington's chorea.

MATERIAL

Tigloidine is a rare and irregular component of *Duboisia* alkaloids. The naturally occurring alkaloid is the tiglic ester of pseudo-tropine. This compound was used in most of the cases treated. Supplies, in tablets of 10 and 20 milligrammes, were obtained by the courtesy of T. & H. Smith, Edinburgh. In a few instances the tiglic ester of tropine was used. So far no difference of action could be observed. Neither of the compounds caused untoward side effects, nor did there seem to be any difference in the dosage required. In the present text the name tigloidine is used for either of the compounds.

RESULTS

Owing to the short and often irregular supply of the drug only a limited number of patients (12) could be treated. To each patient selected was allotted an amount of drug sufficient for a trial and, if possible, for a re-trial after an interval of several weeks or months. In several cases calcium lactate or starch tablets of identical

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appearance were given before the actual trial, or during the interval.

Trials on early, ambulant subjects of Huntington's chorea failed to give clear-cut results. Most of these patients showed gross emotional instability and lowered resistance to environmental stress. The assessment of the therapeutic effect was rendered more difficult through the frequent contradiction between the statements of the patient and those of the relations. It would almost appear as if, in the early stages of the illness, the extent of the instability of the patient was greater than the range of the corrective influence of the drug.

The state of the more advanced hospitalized patients was more stable and showed under irritating experiences, or under intensive placebo medication, no fluctuations comparable in degree to those exhibited in the earlier cases. Several patients with well developed Huntington's chorea were not treated at all. These, as well as the other patients during the intervals of tigloidine treatment, were used as controls.

The observations were directed towards recording the incidence and the extent of changes in motor coordination, in ward behaviour as regards personal conduct as well as attitude towards attendants, visitors and other patients, and in mood and general emotional outlook. The results are summarized in Table I. All of these patients were hospitalized, and most of them were well adjusted to hospital routine when the treatment was started; in a few cases treatment was continued after their release from hospital.

It will be seen that, even on prolonged medication, no therapeutic effect was observed with daily doses below 80 milligrammes of the drug. The effective dose varied with the patient, usually between 120 and 180 milligrammes per day. Once a good response was obtained, further increase of the medication, even to 300 or 400 milligrammes per day, did not further increase the effect. The drug showed thus a very narrow range of action: the beneficial effects manifested themselves when a

TABLE I.

Observations Made in Trials with Tigloidine

Name[1]	Sex	Age (Years)	Duration[2] (Years)	Treatment		Improvement[3]		Remarks
				Milligrammes per Day	Months	Motor	Mood	
T.B. ..	Male	47	4	120	6	+++	++	Released from hospital. Euphoric
				120	8+12	+++	++	Retrials
				240	3	0	0	Retrial after two years
W.C. ..	Female	50	2	120	3	+	+++	Lost lachrymose depression
				240	3	+	+++	No further improvement
F.I. ..	Male	46	7	120	1	+	+	Less hostile
				240	2	++	+	Motor improvement only
				360	2	++	+	No further improvement
M.K ..	Female	47	11	60	1	0	0	No change
				120	1	+++	+++	Euphoric and amiable
				120	2+3	+++	+++	Retrials
				360	1	0	0	Retrial after two years
B.L.* ..	Female	41	6	120	3	+	++	Euphoric
F.L.* ..	Female	56	5	60	2	0	0	No change
Y.L.* ..	Female	52	12	120	1	0	0	No change
L.O.* ..	Male	60	6	120	1	0	0	No change
R.V. ..	Female	55	8	60	1	0	0	No change
				180	2	+	++	Mainly mood improved
M.W.* ..	Female	74	12	60	1	+	?	Slight improvement
H.W.* ..	Male	52	6	180	1	0	0	No change
V.Z. ..	Female	52	6	120	2	+	++	Distinctly improved
				240	4	+++	++	Released from hospital

[1] The initials are arbitrary and do not refer to the actual name of the patient. All subjects have a family history of Huntington's chorea except W.C., whose father could not be traced. In patients marked with an asterisk, trials with higher doses could not be made owing to technical difficulties or to temporary shortage of the drug.

[2] Duration of the illness in years since the time when it seriously interfered with professional or social life.

[3] "Motor" includes the ability to dress, eat, articulate, write, etc. "Mood" includes emotional stability, manageability and attitude to environment. No significant changes were observed in higher intellectual faculties such as memory, insight or judgement. "Amiability" accompanying improvement in mood was pronounced in several cases; it appeared to be due to a facilitation of contact rather than to a loss of moral standards.

"+"=Slight though significant improvement. "++"=Marked improvement. "+++"=Mood—change from depression towards normal or euphoria. Motor—a reduction of choreic movements to a degree no longer interfering with activity. (In some cases to a degree enabling the patient to be released from hospital and return to his occupation.)

certain dosage was reached, and became optimal on a further increase of the medication by 10 to 20 milligrammes per day. In no case could all symptoms of the disease be abolished, and none of the remaining symptoms were affected by doubling or trebling the daily dose. No toxic symptoms were observed amongst our patients during six to eight weeks of constant maintenance medication.

Once optimal improvement had been reached and maintained for several weeks, the drug was discontinued or replaced by placebo. The patients relapsed within a fortnight to their previous condition, but when four to six weeks later the treatment was re-started, the same extent of improvement was effected by the same dose. No tolerance was observed; the effective dose remained unchanged as long as the clinical picture of the illness remained the same. The case of Miss M. K. will be noted: the patient showed on several re-trials conducted after two to three months' interval the same almost dramatic improvement. Two years later, when her clinical condition had much deteriorated, no beneficial response could be obtained even with increased medication. Her case, together with two others, is presented in detail to illustrate the therapeutic procedure and the pattern of the clinical changes.

Case Histories

Case 1.—Miss M. K., aged 47 years, is a member of a family suffering from Huntington's chorea. Her mother, her two sisters and one brother of her mother are suffering from the disease. The patient was well and active till the age of 36 years, when she began to show attacks of depression and outbursts of temper. Slight involuntary movements of the neck, forearms and legs appeared at the same time. Her condition deteriorated progressively; four years later she had to be transferred to a hospital, where she has remained ever since.

When first treated with tigloidine, she was aged 44 years. She suffered from almost incessant gross choreiform movements of body and limbs to the extent that she could not support herself on her legs, threw arms and legs about while sitting, could not dress herself, and had to be fed because she could never reach her mouth with the spoon. In her relationship to attendants or other patients she was irritable and hostile or offensive; she would push or kick them, or grab their food or other belongings from them. Her speech was almost unintelligible, and, from what could be gathered from it and from her responses to questions and suggestions, her intellectual level was very low; she appeared to be almost demented.

She was started on treatment with 60 milligrammes of tigloidine hydrobromide per day, given as two tablets of 10 milligrammes three times a day after meals. This dosage, given over a fortnight, failed to improve her condition. When the dose was increased to 80 milligrammes per day, the patient showed within five days a distinct reduction in the sweep of the choreiform movements to the extent that she could sit without danger of rolling off the chair. At the same time she became more friendly, and was more amenable. A few days later she was able to eat at the table with fairly good coordination, and she no longer grabbed food from her neighbour's plates. After a further week she was able to dress herself; she walked with more confidence and moved spontaneously about. Her speech was less slurred and quite intelligible, she was more cheerful, and she stated spontaneously that she felt much better. She took an interest in her surroundings, but was inclined to be over-talkative. Occasionally she was euphoric and amiable, clinging on to the nurse or doctor. Her intellectual level, however, did not seem to be much improved. She answered simple questions and was aware of her surroundings, but it appeared that she could not discuss her own history or any past events. This improvement was maintained as long as the medication was continued. When, after eight, weeks, the drug was replaced by placebo, the patient began to deteriorate in her choreiform movements as well as in her mood, reaching within about ten days her previous condition. When the medication was re-started, she reached within about the same period the level of her previous improvement. This sequence was observed three times within the following year. Once the medication was increased to 120 milligrammes per day. The only effect was that the patient became voluble, amiable and euphoric; neither her control of movements nor her intellectual performance was improved above the extent reached with 80 to 100 milligrammes per day.

The medication was finally stopped because the supply of the drug was exhausted. When, two years later, tigloidine became again available, the patient, who had further deteriorated in the meantime, showed no response whatever, even when the daily intake was increased to 200 and 300 milligrammes per day.

Case 2.—Mr. T. B., aged 47 years, comes from a family suffering from Huntington's disease. Four of six siblings of his mother's generation and three out of five of his own were afflicted with the disease. When first admitted to hospital, three years ago, he was in a state of gross excitation, restless and overactive, noisy and troublesome, sometimes elated, sometimes depressed. He was making constant involuntary purposeless movements of his face, neck, body, arms and legs. Those movements were so severe that he threw the bedclothes off the bed and was unable to stand and to do anything like washing and dressing himself or eating. He was inclined to be incontinent of fæces and urine; his speech was very slurred, and sometimes almost unintelligible. On examination he was found to be deluded and barely aware of his surroundings; his stream and content of thought were vague, rambling, incoherent and often meaningless.

The patient was a trained mechanic and had worked for 30 years with the same firm. Four years prior to coming under observation he became emotionally unstable, showing periods of depression or outbursts of rage. At the same time the movements of his hands became uncertain and inclined to be exaggerated. This motor impediment increased during the following two years to the extent that he was transferred to the store. Four months before his admission to hospital he had to give up work, being neither manually nor mentally able to continue with his duties as assistant storekeeper. At home, the main difficulty was presented by his frequent emotional outbursts; at the height of one of these tantrums he was transferred to hospital. After a few days of observation, during which time the patient calmed considerably without, however, losing his excessive choreiform movements, the patient was started on treatment with 120 milligrammes of tigloidine hydrobromide per day, given as two tablets of 20 milligrammes three times a day after

meals. After one week he had became fully continent of urine and fæces, and was able to get out of bed and, if staggeringly, to walk about. His delusions were reduced, and he showed some insight into his condition; he was distinctly less lachrymose and less depressed. During the second week of treatment there was a further progressive diminution in his choreiform movements, and he became more cheerful and hopeful in his outlook. His memory had returned, and he was able to converse more freely and clearly, with full insight into his illness and into the history of its development; he was fully orientated as to time and place and able to discuss sporting or world news. In the third week he had improved to the extent that he could perform fine movements, such as rolling his own cigarettes or writing, although his script was rather shaky, with big, childlike letters, though of equal size, and with approximately equal spacing between the words. He was slightly euphoric, but his mood was stable; he was good-tempered and not easily annoyed. One week later he could be released with the instruction to continue the medication and to present himself once per week for examination.

At home the patient maintained his improvement. His wife stated that he was giving no trouble, and was considerably easier to manage than before his admission to hospital. Two months after his release from hospital he considered himself well enough to try to return to his old job. A few weeks later he started working and was capable of fulfilling his duties. Whenever, through shortage of the drug or through negligence, he stopped the intake of tigloidine, he became, according to his wife, emotionally unstable within about one week, and his choreiform movements increased. On re-introduction of the medication his condition improved again within about the same period. This response was seen on several occasions over the following two years, during which time the patient continued to work in his job. At no time was it necessary to increase the medication, and at times the patient felt so much in control of his movements that it needed persuasion to prevent him from driving his car.

Towards the end of the second year the medication began to lose its effect. The patient became moody and irritable with outbursts of temper to the extent of threatening or attempting violence. He could still do his job, but his wife found it more and more difficult to live with him. Doubling the medication (to 240 milligrammes per day) appeared slightly to improve his movements, but it failed to control his mood and his temper. During one of these crises his wife had him readmitted to hospital. It was no longer possible to improve his condition to the extent that he could be allowed to be at large.

CASE 3.—Mr. F. I., aged 46 years old, a glass cutter, came from a family afflicted with Huntington's chorea. At the time, his father was still alive and bedridden from the disease. The patient had noted the first symptoms of the illness seven years previously; they consisted of slight, involuntary movements of his hands and of his neck. The disturbance progressed, and two years later he had to give up his job. At about the same time he began to show a change of mood, insofar as he became irritable and argumentative, and was constantly "on edge". During the next few years his choreiform movements became more violent, but he was admitted to hospital mainly because he had become unmanageable at home, displaying strong emotional outbursts with threats of violence and increasing hostility to his family, mainly his daughter. When considered for treatment, he had become unable to dress himself and was just able to eat. His gait was unsteady and staggering; even at rest he showed frequent widely sweeping movements of his arms and legs. He was a heavy smoker, but usually he left the cigarette dangling from his lips because he was unable to replace it once he succeeded in removing it. He was very conscious of his illness and usually kept his hands stiffly in the pockets of his tightly buttoned jacket to restrain the movements. He was asocial and hostile. His speech was slurred and often he refused conversation. He was inclined to take exception and offence, and there were frequent complaints in the ward about his interference and aggressive behaviour. He looked forward to the visits of his daughter, but mostly they ended in an argument. His memory and his intellect were, as far as could be ascertained, unimpaired.

The patient was started on treatment with 50 milligrammes of tigloidine per day, and this dose was within a fortnight gradually increased to 120 milligrammes per day, when the patient complained about mild giddiness, tiredness and somnolence. The dose was reduced to 80 milligrammes per day, and then within one week again increased to 100 and finally 120 milligrammes per day. This dose was then tolerated without any unpleasant side effects. Maintained on this dose, the patient showed distinct improvement of his choreiform movements, which diminished in extent, violence and frequency. He became able to dress himself and to eat without difficulty. He smoked, holding the cigarette in his hands. His walking had improved and, curiously, was greatly steadied if somebody, even lightly, placed a hand on his shoulder. His speech was more distinct, and he was, if not exactly friendly, at least polite and willing to conduct a conversation. He appeared to be less argumentative, and there were fewer complaints about him in the ward. Also he no longer argued with his daughter when she visited him. The patient retained this level of improvement during the whole period he was maintained on 120 milligrammes of tigloidine. When, after three months, the medication had to be stopped because of shortage of supplies, he relapsed within a few weeks to his previous condition.

DISCUSSION

It must be emphasized that it is not intended to present tigloidine as a cure for Huntington's chorea. The observations gave no indication that the drug could remedy the existing damage to the nervous tissue, or that it could arrest the further progress of the illness. They showed only that, in some cases, and apparently only at certain stages of the disease, the drug temporarily reduced the extent of symptomatic disturbances. This action was twofold: it could be exerted on the frequency, range and violence of the choreiform movements, or it could manifest itself in an improved mood and increased emotional stability of the patient. Varying from case to case, one or other of these two effects was more pronounced.

The action of low concentrations of atropine, yohimbine and several other alkaloids on the skeletal nerve and muscle of the toad produces a sort of quick fatigue on repeated stimulation which has been described as "delayed recovery" (MacCallum, McCallum and Shaw, 1949). Since tigloidine, though lacking the parasympathetic-olytic effects of atropine, causes under the same conditions this same effect, some action of the

drug on skeletal nerve and muscle could be expected. It was expected that a peripheral depressant effect exerted on the neuro-muscular system would result in a sort of fatigue, in an over-all increase in threshold, and thus in a cessation of responses to weak and rapidly repeated stimuli. Beneficial effects on tremors and rigidity were in fact observed in a few cases of Parkinsonism with the use of moderate doses of the drug, 90 to 150 milligrammes per day.

When in some cases the daily dose was increased to 400 and up to 600 milligrammes per day, it was very surprising that there was never any flaccidity or incipient paralysis of skeletal or respiratory muscles. In fact, voluntary movements were affected neither in their release nor in their execution. All motor effects observed, whether they consisted only in slight improvement or whether they were dramatic, involved merely the frequency and the extent of involuntary movements, resulting in improved speech, in improved postural integration and in better coordination of purposeful movements. The drug thus exercised a distinct central effect on disturbances of the extrapyramidal system, without noticeably affecting voluntary movements or vegetative functions.

The extent of these effects was not necessarily related to the gravity of the symptoms. Miss K., who when first seen had to be classed as rather advanced, experienced a striking recovery, while other patients, who were clinically in a much better condition, did not show any noticeable benefit. With regard to the progress of the illness, Mr. B. kept his improvement for almost two years, remaining able to do his job and to circulate freely in the community; then he developed within a few weeks a degree of emotional instability which could no longer be controlled by the drug and necessitated his admission to hospital. It seemed in general that grossly deteriorated patients did not respond to the drug, but it was not possible to predict which patients would respond or the extent of the improvement. Brock (1953), discussing the mechanism of extrapyramidal symptoms, states:

> Loss of the small internuncial cells, prominent in the striatum, is said to play a part in evoking choreiform movements . . . It must be borne in mind that the active signs produced by destruction of cells, point to the fact that other cell groups are either released from inhibitory influences, or abnormally excited. Hence, involuntary movements and tonus changes noted in disease of the striatum may arise from altered activity of the cells of the pallidum.

Tigloidine may not act on the sites of the primary disturbances causing the disease, but may act only as a depressant on the "altered activity" of secondary sites. This assumption of a localized action of the drug would be in accordance with the observations that (i) moderate daily doses had no effect whatsoever, (ii) the lowest dose which had any effect was very near (within 10 to 20 milligrammes) to the dose which gave optimal effect, (iii) any further increase of the daily dose did not cause further improvement, and (iv) in all cases which responded the improvement could be brought only to a certain level, but no further. It would seem that optimal beneficial effect was reached when the drug was given in a dosage sufficient to "saturate" certain sites, but that the drug did not affect the whole extent of the pathological disturbance.

A beneficial effect on mood and emotional outlook can obviously be caused by any therapy which improves disturbing somatic symptoms. It did not, however, appear that in the individual cases of Huntington's chorea treated with tigloidine, the two effects were necessarily connected and parallel. In the case of Mr. I., the motor control was considerably improved, but it was doubtful whether his better manageability and lowered irritability were due to a direct effect of the drug, or merely to the absence of the annoying excessive and grotesque movements. On the other hand, Mrs. C., who displayed only slight disturbances of coordination and was apparently unaware of occasional involuntary movements of her neck and hands, gained only slight, if any, improvement of her motor control, but her lachrymose suicidal depression gave way to a more contented, even slightly euphoric outlook. In other cases the character of the mood changes was out of proportion to the clinical condition or the mental awareness of the patient. Mr. B. exhibited an almost shocking degree of levity and jocularity while perfectly rationally discussing the early symptoms of Huntington's chorea developing in his younger brother. Mrs. K. became overwhelmingly affable and amiable, but her mental alertness and level of functioning remained unchanged at a very low level; possibly she was not even aware of the improvement in the control of her posture and movements. It seemed that the improvement in mood was due to an independent central action of the drug and was not secondary to the achievement and realization of motor improvement.

On these considerations trials with the drug are being extended to cases of endogenous depression as well as to cases of disturbances of motor regulation (post-traumatic spinal spastic paraplegias) (Gershon, Shaw and Trautner). In both conditions good results have been recorded, and in several instances the drug brought relief

when the usual medication had failed; a full record of these observations will be presented in a following publication. The results seem to confirm the assumption of an independent action of the drug on mood and on motor coordination.

Chemically tigloidine is a close homologue of atropine. Atropine is an ester of the aminoalcohol tropine with tropic acid. Both components by themselves have low toxicity and little pharmacological action. The linking of the two by an esterlink renders the compound highly active.

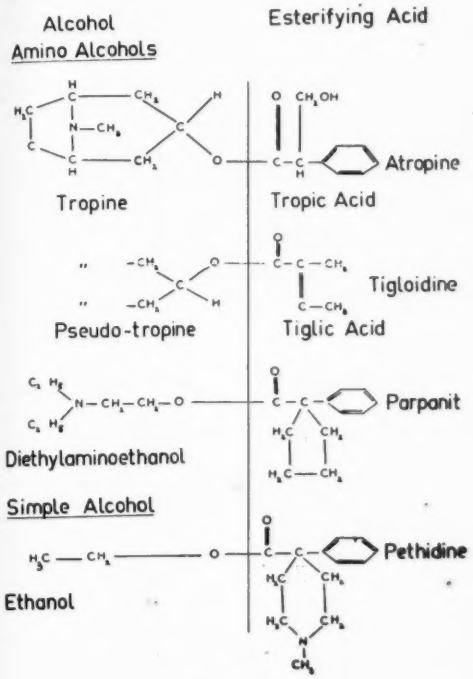

FIGURE I

Formulæ of tigloidine and related compounds referred to in text

The parasympathicolytic action of atropine and its homologues is to a large extent dependent of the presence of an alcoholic group and of a ring structure in the molecule of the esterifying acid. The benzoic acid ester has mainly local anæsthetic properties. The esters of saturated aliphatic acids show no clear-cut pharmacological action. Tiglic acid is an aliphatic acid; it contains neither an alcoholic group nor a ring structure in its molecule, and it is therefore not surprising that it lacks most of the properties of atropine, particularly the parasympathicolytic effects shown by homatropine and related compounds. But tiglic acid is unsaturated, and it is possible that this alpha-beta-unsaturation is responsible for its pharmacological action on the nerve-muscle preparation, on the methedrine-overactive dog and on the clinical symptoms described. So far very few alpha-beta-unsaturated acids have been incorporated into pharmacological compounds.

A further structural property characterizing tiglic acid, and possibly partly responsible for the action, is the fact that the alpha carbon atom has no hydrogen substitution. Its four valencies are linked with carbon, as they are in "Parpanit" and in pethidine (see Figure I). The former, an ester of diethylamino-ethanol, is recommended for the treatment of Parkinsonism; pethidine, which is esterified with ethanol and has the amino-group in the piperidine moiety of the acid, exhibits analgesic, spasmolytic and euphoric effects. Considering the esterified aminoalcohol, it may be mentioned that tropine contains amino-propanol incorporated in its ring structure, and reserpine, which has been shown to have a similar effect to tigloidine on the symptoms of Huntington's chorea (Bower, 1957), contains amino-butanol, which shares the amino group with a tryptamine moiety incorporated into the same ring structure. The site of action of these compounds may be suggested by the observation that reserpine, though it improves choreiform symptoms in some patients, causes Parkinsonism-like symptoms in others.

It will be of interest to follow in further investigations the suggestions arising from the similarity of these structures, particularly in view of the fact that modern pharmacology supplies the psychiatrist with a variety of rather unspecific sedatives and hypnotics as well as of more or less successfully controllable physiologically active amines, but it offers very few anti-cholinergic compounds which are sufficiently non-toxic and selective in their action to alow their prolonged use in disorders which may result from the overactivity of central cholinergic transmissions.

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ABDOMINAL PSEUDO-DISTENSION WITH PAIN, DYSPNŒA AND DYSPHAGIA IN RELATION TO DISTURBED DIAPHRAGMATIC MOVEMENT[1]

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SUMMARY

The mechanism of abdominal pseudo-distension was studied in two hysterical patients and in four apparently normal adult males who could simulate the hysterical condition at will.

It was concluded that the principal factor causing abdominal pseudo-distension was substantial descent of the diaphragm caused by its own active, sustained contraction; this produced marked increase of abdominal girth and of intra-abdominal pressure, whether exaggerated lumbar lordosis was present or absent. Exaggeration of lumbar lordosis as an isolated factor, far from producing abdominal pseudo-distension, caused no increase of abdominal girth and no significant increase of intra-abdominal pressure. Exaggeration of lumbar lordosis, however, increased slightly the convexity of the anterior abdominal profile. In the four apparently normal males, the increase of abdominal girth attainable by voluntarily forcing down the diaphragm was actually reduced by exaggeration of lumbar lordosis, probably because such lordosis also tended to make the anterior abdominal wall taut.

Reasons are given for preferring the term "abdominal pseudo-distension" to other terms.

When the diaphragm shows such sustained contraction in hysteria, pain, dyspnoea and a type of dysphagia may occur; these are discussed.

IN 1838 Richard Bright described a hysterical woman whom he had seen in 1824 with a "swollen abdomen", previously operated upon for "ovarian tumour"; at operation the surgeon "finding that there was no tumour, brought the wound together". Bright noted, post-operatively, that the patient still suffered from "puffing up of the abdomen". During the next hundred years a number of authors, including Osler (1904), wrote of phantom tumour and of apparent distension of the abdomen in hysteria.

More recently Purves-Stewart (1945) has written on the subject, followed by Alvarez (1949; 1951), Roussak (1951), Westdahl (1951), Schopbach, *et alii* (1952), Stewart and McConnell (1953) and Ewing and Millar (1955).

A century and more after the unhappy experience of Bright's hysterical patient, hysterical pseudo-distension of the abdomen continues its deceptive simulation of organic intra-abdominal conditions. Alvarez (1949) described two patients with six and one patient with five previous laparotomies, commenting on the uselessness of operations designed to cure "hysterical abdominal bloating". Two patients described by Roussak (1951) found their way to an operating theatre: one had a "normal appendix" removed, the other was returned to the ward without laparotomy after general anæsthesia caused the apparent distension to subside. The patient described by Westdahl (1951) had been operated upon 12 times, at different hospitals, for almost identical episodes of "pseudo-bowel obstruction"; peritoneal adhesions following the numerous exploratory operations finally caused organic intestinal obstruction. Because of apparent abdominal distension, the patient described by Ewing and Millar (1955) had a laparotomy, but no intestinal obstruction was found. The first patient described hereunder beguiled his way to an operating theatre in 1957, but was returned to the ward without laparotomy when general anæsthesia dissipated the abdominal pseudo-distension.

The following study is an attempt to analyse further the mechanism of abdominal pseudo-distension in two hysterics and in four apparently healthy, adult males who could simulate the condition at will.

REPORTS OF CASES

CASE 1.—A man aged 41 years, by occupation a fencer of farm properties, stated that he was knocked momentarily unconscious by a branch of a falling tree in May, 1957. He said that he was able to rise to his

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feet within a few minutes of the injury and drive a truck five miles to his home. He stayed in bed for three days but felt unable then to return to work, because on attempting to walk and exert himself, throbbing headache, a feeling of nausea and general weakness and a tendency to faint compelled him to rest. Six weeks after the injury he was referred to the Brisbane Hospital for investigation of post-traumatic headache.

FIGURE IA

Case I.—Showing hysterical pseudo-distension of the abdomen. Abdominal girth, 91·5 centimetres. Abdominal wall tense and firm to palpation. (End of a quiet inspiration)

FIGURE IB

Case I.—After induction of light general anæsthesia. Abdominal girth, 81 centimetres. Abdominal wall soft to palpation. (End of a quiet inspiration)

Physical examination then revealed slight general weakness of the right upper and right lower limbs though the patient was apparently unaware of this. The deep reflexes were normal, but the plantar responses were equivocal. A diagnosis of subdural hæmatoma was considered but plain X-ray films of the skull, examination of the cerebro-spinal fluid and bilateral carotid angiograms showed no abnormality. Pneumoencephalography revealed slight dilatation of the left lateral ventricle and slight excess accumulation of air over the surface of the left cerebral hemisphere, the appearances suggesting a minor degree of left cerebral atrophy. This was consistent with an organic basis for the slight right hemiparesis, but it was considered that the lesion was most likely of long standing and not related to the present complaints. The patient was in receipt of compensation for the headache.

On the second evening after the pneumoencephalography, after being told that he could be discharged from hospital, the patient began to complain of severe pain around the navel and a feeling of abdominal tightness and discomfort; he complained also of nausea but did not vomit. He stated that he had passed a normal bowel motion that morning. On examination, the abdomen appeared very distended and tense, was slightly tender to pressure and was generally tympanitic to percussion. Peristaltic sounds were heard normally, and rectal examination revealed an empty rectum but no abnormality. Plain X-ray films of the abdomen

indicated rather more gas than usual in both small and large bowel but no fluid levels. A Ryle's tube was passed into the stomach, which was aspirated every half hour, normal gastric contents being obtained. By 11.30 p.m. that night the abdominal pain and appearance of abdominal distension had increased, and the surgeon on night-duty considered it safest to prepare for laparotomy in view of probable intestinal obstruction. However, on induction of general anæsthesia by intravenously injected sodium thiopentone, the abdomen rapidly regained a normal appearance, and the surgeon referred the patient back to the medical ward without operation. On recovery from the general anæsthesia the apparent abdominal distension returned, and it persisted when the patient was awake, but was observed to disappear during sleep. After the first 48 hours of abdominal pseudo-distension he no longer complained of severe abdominal pain but only of moderate abdominal discomfort; he was able to eat meals normally and had normal bowel evacuations.

The abdominal pseudo-distension appeared to be a "conversion symptom". On sympathetic questioning the patient disclosed panic at being sent home, about a hundred miles from the nearest doctor. He feared that he might have further fainting attacks when truck driving, and that one of these might cause an accident killing his wife and children. He had previously concealed the fact that he had accidently killed a child who had fallen off the back of his truck and been crushed by the truck's wheels. This event still caused nightmares and troubled his waking thoughts. The past history revealed that he was sometimes away from work receiving compensation for several months after minor as well as major injuries. In 1940, after convalescence in hospital from a fractured pelvis, abdominal pseudo-distension had kept recurring for three months; return to work, then imminent, had been postponed.

To investigate the mechanism of the abdominal pseudo-distension the abdomen was photographed, and X-ray films were taken: anteroposterior films of the abdomen and chest, and lateral films of the thoraco-lumbar spine, each exposure being taken firstly at the end of a quiet inspiration and secondly at the end of a quiet expiration; the patient lay supine for all these procedures. An injection of 0·5 gramme of sodium thiopentone was then given intravenously (0·4 milligramme of atropine sulphate had been injected hypodermically one half hour previously; the patient weighed 72·5 kilogrammes). Coincidently with the induction of light general anæsthesia the abdominal pseudo-distension subsided rapidly over a period of a few seconds without detectable escape of flatus (see Figures IA and IB). The abdominal girth at umbilical level changed from 91·5 centimetres to 81 centimetres, (readings at the end of a quiet inspiration), and the anterior abdominal wall, previously stretched and tense, became quite soft to palpation. Repetition of the radiographs indicated that after induction of the light general anæsthesia the diaphragm had ascended approximately 7·4 centimetres (the top of the right

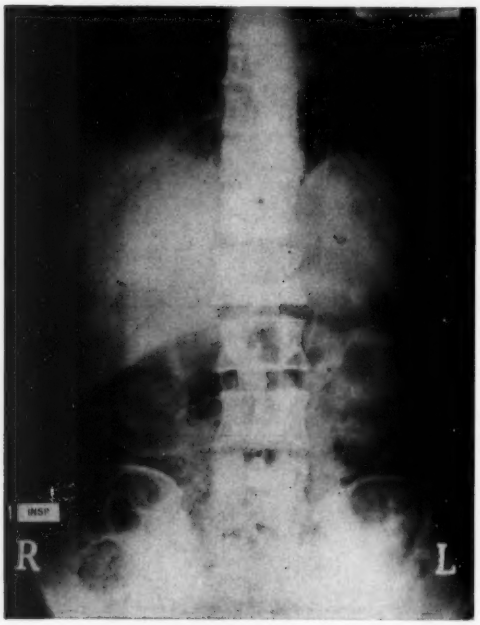

FIGURE IIA

Case I.—Radiograph of abdomen in the stage of hysterical pseudo-distension. (Patient supine, at the end of a quiet inspiration)

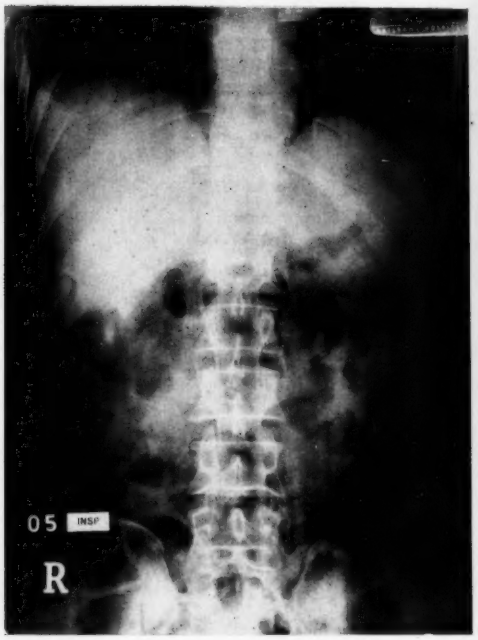

FIGURE IIB

Case I.—Radiograph of abdomen after induction of light general anæsthesia; the domes of the diaphragm have risen a mean distance of 7·4 centimetres. (Patient supine, at the end of a quiet inspiration)

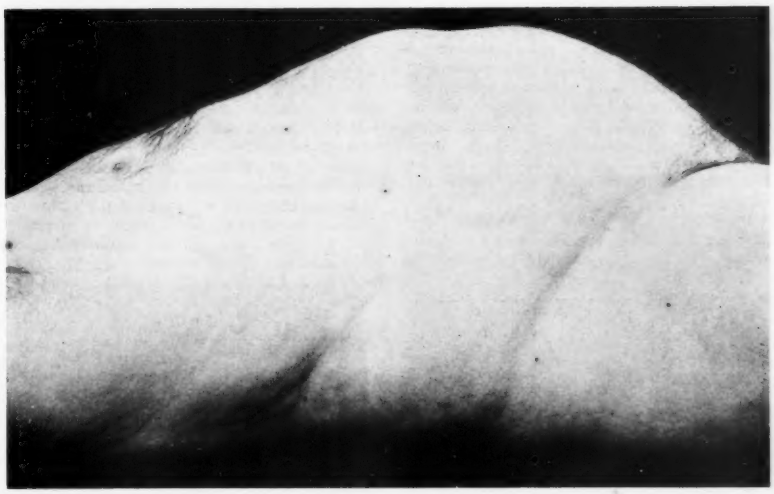

FIGURE IIIA

Case II.—Showing hysterical pseudo-distension of the abdomen. No significant lordosis present. Abdominal girth, 119·5 centimetres. Abdominal wall tense and firm to palpation. Note that the antero-posterior diameter of the chest is less than in Figure IIIB. Chest circumference at nipple level, 101·5 centimetres. (End of a quiet expiration)

FIGURE IIIB

Case II.—After induction of light general anæsthesia. Abdominal girth, 108 centimetres. Abdominal wall soft to palpation. Chest circumference at nipple level, 105·5 centimetres. (End of a quiet expiration)

cupola had ascended 7·5 centimetres, that of the left 7·2 centimetres; see Figures IIA and IIB). The degree of lumbar lordosis was unchanged.

It thus seemed that sustained contraction of the diaphragm was responsible for the abdominal pseudo-distension. During the phase of abdominal pseudo-distension radiographs revealed a respiratory excursion of the diaphragm of 0·5 to 1·0 centimetre.

To the accompaniment of persuasion, explanation and suggestion, the abdomen gradually returned to normal during waking hours, and within two weeks the patient was discharged from hospital. He returned to work, and when seen nine months later he said that he felt perfectly well and had had no recurrences.

CASE 2.—In February, 1957, a man aged sixty years, of no fixed employment, complained of nausea and

abdominal pain increasing in severity over three days, and during this time he vomited all food given. Since 1919 the patient had suffered from abdominal symptoms of many sorts, but especially pain and discomfort in various parts of the abdomen; between 1919 and 1937 he had had appendicectomy, cholecystectomy and an operation for alleged left renal calculus. Two further laparotomies had been performed for "adhesions". From 1955 to 1957 he had complained increasingly of attacks of abdominal distension and swelling.

On examination the abdomen appeared very distended and tense; it was generally tympanitic to percussion and particularly tender at the site of the

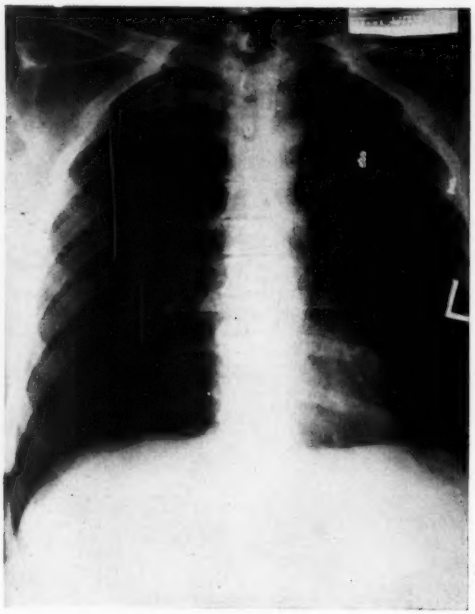

FIGURE IVA

Case II.—Radiograph of chest when abdomen was showing hysterical pseudo-distension. (Patient supine, at the end of a quiet expiration)

cholecystectomy scar. The bowels had not been open for six days, though the patient stated he had been subject to lesser degrees of constipation for many years. A diagnosis of intestinal obstruction was seriously considered; however, plain X-ray films of the abdomen did not show undue accumulation of gas in the bowel, and no fluid levels were seen. There was no fever or blood leucocytosis. The patient was treated conservatively, and a Ryle's tube was passed into the stomach, which was aspirated at regular intervals, sweetened drinks being given by mouth after each aspiration. After a few days the nausea, vomiting, abdominal pain and appearance of abdominal distension subsided, and the Ryle's tube was withdrawn. This was followed by prompt return of the abdominal symptoms and signs, relief again being afforded by insertion of the Ryle's tube as before. It was nine weeks in all before the Ryle's tube could be withdrawn without marked recurrence of the abdominal symptoms and signs. During this period the constipation was alleviated by enemas administered once or twice weekly.

In August, 1957, the patient was referred to the Brisbane Hospital for further investigation. He was still liable to episodes of abdominal pain, discomfort and apparent distension, these lasting from several hours to a few days. Strong passive flexion of the hips did not abolish the abdominal pseudo-distension.

The mechanism of the abdominal pseudo-distension was investigated as in Case I, with similar premedication. An injection of 0·6 gramme of sodium thiopentone was given intravenously (the patient weighed 89 kilogrammes). Again, coincidently with the induction of light general anæsthesia, the abdominal pseudo-distension rapidly subsided over a period of a

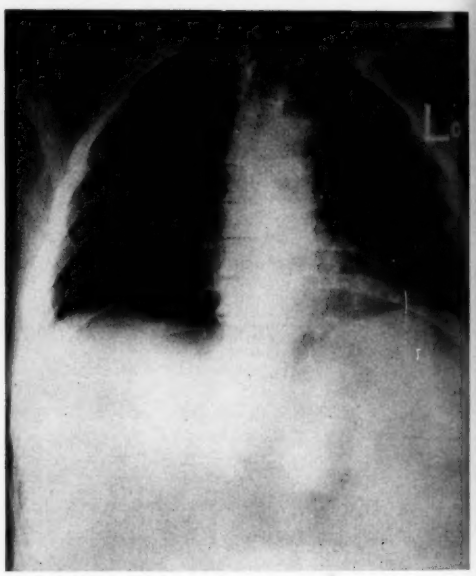

FIGURE IVB

Case II.—Radiograph of chest after the induction of light general anæsthesia; the domes of the diaphragm have risen a mean distance of 7·9 centimetres. (Patient supine, at the end of a quiet expiration)

few seconds without detectable escape of flatus (see Figures IIIA and IIIB). The abdominal girth at umbilical level changed from 119·5 centimetres to 108 centimetres (readings at the end of a quiet expiration), and the anterior abdominal wall, previously stretched and tense, became quite soft to palpation.

Repetition of the radiographs showed that the diaphragm had ascended approximately 7·9 centimetres (the top of the right cupola ascended 7·2 centimetres, that of the left 8·5 centimetres; see Figures IVA and IVB). During the stage of abdominal pseudo-distension, contraction of the lower part of the thorax was coincident with the apparent abdominal distension. Figures IIIA and IIIB show that the antero-posterior diameter of the lower part of the thorax during the phase of abdominal pseudo-distension was less than when the abdomen had subsided after induction of light general anæsthesia. At these times

the chest circumference at nipple level was approximately 101·5 centimetres and 105·5 centimetres respectively. (Both photographs and both measurements were taken at the end of a quiet expiration.) When the thoracic vertebrae of the two series of lateral X-ray films of the spine were superimposed, it was seen that the third and fourth lumbar vertebral bodies before induction of general anæsthesia were about eight millimetres more anteriorly placed than the third and fourth lumbar vertebrae after induction of general anæsthesia ; thus only a very trivial increase of lumbar lordosis was present during the phase of abdominal pseudo-distension.

FIGURE VA

Case II.—Control radiograph (trunk vertical) 60 seconds after the taking of the first mouthful of 70 millilitres of barium sulphate cream. Note free passage of opaque medium to stomach and the absence of any "normal œsophageal ampulla"

It therefore appeared that sustained contraction of the diaphragm caused the abdominal pseudo-distension. During the phase of abdominal pseudo-distension the diaphragm showed a respiratory excursion of 0·5 to 1·0 centimetre.

The patient also complained spontaneously that when his "belly was distended" he had trouble swallowing his food, which seemed to him to stick near the lower end of his breast bone and added to his abdominal discomfort ; he also complained of difficulty in breathing. The complaint of dysphagia was investigated as follows : 70 millilitres, constituting half of a prepared mixture of "barium sulphate cream" (as used for radiological examination of the œsophagus in the Brisbane Hospital), were swallowed within a period of 35 seconds distributed over five spoonfuls ; from the first mouthful X-ray films of the chest were taken every 30 seconds for three minutes. Twenty minutes later a check X-ray film showed that the œsophagus was completely free of X-ray opaque material. An attack of apparent abdominal distension was next induced by suggestion and subcutaneous saline injection ; the other half of the prepared mixture of "barium sulphate cream" was then swallowed in precisely the same way and its passage examined radiologically exactly as before.

Comparison of the two series of films revealed that, as compared with the initial control series, a portion of the swallowed "barium sulphate cream" was

FIGURE VB

Case II.—During stage of hysterical pseudo-distension of the abdomen. Radiograph (trunk vertical) 60 seconds after the taking of the first mouthful of 70 millilitres of barium sulphate cream. A portion of the opaque medium remains in the lower part of the œsophagus. The patient complained of dysphagia

unduly retained in the lower part of the œsophagus during the phase of abdominal pseudo-distension (see Figures VA and VB) ; this was so in each of the six X-ray films taken over the three-minute period and appeared to be a physiological correlate of the complaint of dysphagia. The whole experiment was repeated one week later with the same results. On another day the patient swallowed a watery suspension of barium sulphate (as used for radiological examination of the stomach and duodenum at the Brisbane Hospital) ; an attack of abdominal pseudo-distension was induced as before and the patient tipped into the Trendelenburg position ; no hiatus hernia could be demonstrated radiologically (see Figure VI).

The sustained contraction of the diaphragm and tense condition of the abdominal wall suggested that the intra-abdominal pressure might be much elevated

during the phase of abdominal pseudo-distension. Therefore a thin-walled rubber balloon (capacity approximately 50 millilitres) was passed into the patient's stomach and connected by polythene tubing (of three millimetres bore) to a water manometer. With the patient supine the intra-abdominal pressure, as reflected by intragastric pressure, was recorded in centimetres of water in relation to the sternal angle. During the initial control period the mean intragastric pressure was 15·3 centimetres of water at the end of

FIGURE VI

Case II.—After swallowing thin barium sulphate paste, an attack of hysterical pseudo-distension of the abdomen was induced by suggestion and subcutaneous injection of saline. The patient was then examined radiologically in the Trendelenburg position, but no hiatus hernia could be demonstrated

inspiration, 13·1 centimetres at the end of expiration (range of variation 13·5 to 17·0 for inspiration, 12·5 to 15·5 for expiration). Abdominal pseudo-distension was then induced by suggestion and subcutaneous saline injection; during this phase the mean intragastric pressure was 79 centimetres of water at the end of inspiration, 67 centimetres at the end of expiration (range of variation 68–95 for inspiration, 53–80 for expiration). In each case the mean cited was taken of 50 consecutive respiratory cycles. Thus the mean rise of intragastric pressure associated with this episode of abdominal pseudo-distension was approximately 59 centimetres of water (44 mm. Hg.).

After one prolonged episode of sustained diaphragmatic contraction, rapid to-and-fro epigastric movements occurred, at rates varying from 120 to 160 excursions per minute. The heart rate was 82 to 86 per minute and appeared to bear no time relation to the epigastric undulations, which were superimposed upon the ordinary respiratory excursions of the upper part of the abdomen. These movements only lasted for about ten minutes, were associated with increase

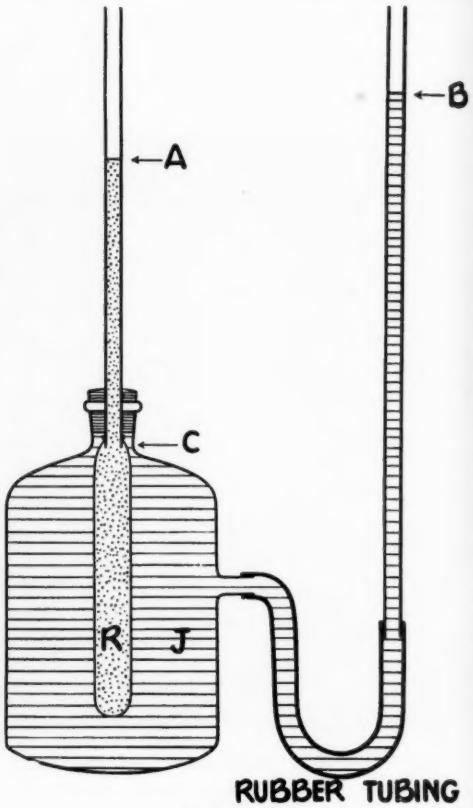

FIGURE VII

A purely physical principle might have been the cause of dysphagia when the intra-abdominal pressure was much elevated in Case II. If the water pressure in the jar *J* outside the rubber balloon *R* was greater than the pressure inside, the balloon collapsed and water would not enter from the column A through the opening at C. (In the diagram the balloon *R* is collapsed, with its front and back surfaces in apposition.) If the pressure outside the balloon was reduced below that inside (as by lowering column B), water flowed freely into the balloon *R* from the column A. (See text)

of pain in the upper part of the abdomen, and were considered to indicate diaphragmatic flutter (Harris, *et alii*, 1954).

The patient had shown various hysterical features for more then forty years. He was invalided out of the Australian Army after minor injury to his jaw in France in January, 1917. An overdependent personality, he earned his own living for periods totalling only fifteen years out of the past forty years. He

TABLE I

Changes of Diaphragmatic Level and Abdominal Girth on Induction of Light General Anæsthesia[1]

Control Subject	Change in Mean Level of Diaphragm with Induction of Light General Anæsthesia (Measurements of Radiographs Taken at End of Quiet Expiration)	Change in Abdominal Girth with Induction of Light General Anæsthesia (Measurements at End of Quiet Expiration)
1. Male, 45 years, 65·5 kilogrammes	Rise of 0·3 centimetre	Unchanged at 78·5 centimetres
2. Male, 57 years, 79·5 kilogrammes	No significant change (fall of 0·1 centimetre)	95 centimetres decreased to 94 centimetres
3. Female, 25 years, 41 kilogrammes	Fall of 0·3 centimetre	Unchanged at 57 centimetres
4. Male, 43 years, 79 kilogrammes	Rise of 0·8 centimetre	86·5 centimetres increased to 87 centimetres
5. Male, 48 years, 77·5 kilogrammes	No change detected	94·5 centimetres increased to 95 centimetres
6. Male, 44 years, 67·5 kilogrammes	Rise of 0·4 centimetre	82·5 centimetres decreased to 81·5 centimetres
7. Male, 49 years, 76·5 kilogrammes	No significant change (rise of 0·1 centimetre)	90 centimetres increased to 91·5 centimetres
8. Female, 26 years, 67 kilogrammes	No change detected	Unchanged at 75 centimetres

[1] Premedication: Atropine sulphate 0·4 to 0·6 milligramme, by hypodermic injection, one-half hour previously. Light general anæsthesia induced by intravenously injected sodium thiopentone 0·3 to 0·6 gramme.

unsuccessfully sought an Australian Repatriation pension on 13 different claims for such conditions as "shell shock", "stomach disease" and neurasthenia. He eventually obtained an invalid pension. In 1957 at the Brisbane Hospital he ably maintained his invalid role with (i) sustained contraction of the diaphragm causing abdominal pseudo-distension, pain, dyspnœa and often dysphagia, (ii) diaphragmatic flutter on one occasion and (iii) a hysterical left hemiparesis and left hemianæsthesia, with classic contradictory features and fluctuations in severity. Analysis of the mechanism of certain hæmodynamic disturbances in this hysteric have also been described (Tyrer *et alii*, in the press).

Attempts at psychotherapy were made before the investigatory studies, but were unsuccessful. In view of the forty years' duration of hysterical features, this was scarcely surprising.

SOME CONTROL STUDIES

Changes of Diaphragmatic Level on Induction of Light General Anæsthesia

Study was undertaken of eight subjects having light general anæsthesia induced for minor surgical procedures unrelated to any abdominal or thoracic abnormality. In the supine position, and at the end of a quiet expiration, abdominal girth at umbilical level and the height of each dome of the diaphragm (measured from radiographs) were recorded before and after the induction of the anæsthesia. The mean level of the diaphragm was taken as the average of the heights of the right and left domes. The premedication and dose of sodium thiopentone were comparable to those employed in studying Cases I and II.

The changes in mean level of the diaphragm were slight and inconstant in direction, and in each case the alteration was less than one centimetre. The changes in abdominal girth were also slight and inconstant in direction, and in no case exceeded 1·5 centimetres (see Table I).

Voluntary Pseudo-Distension of the Abdomen

Four apparently normal adult males (of whom three were medical graduates) could, after practice, produce varying degrees of abdominal

TABLE II.

Abdominal Girth and Lower Thoracic Circumference in Relation to Sustained Diaphragmatic Contraction and Exaggerated Lumbar Lordosis in Four Apparently Normal Adult Males[1]

Control Subject	A. Abdominal Girth (Resting)	B. Abdominal Girth (Maximum Lordosis)	C. Abdominal Girth (Maximum Pseudo-distension)	D. Mean Descent of Domes of Diaphragm to Produce C from Resting Position (Radiographs Compared at End of Quiet Expiration)	E. Abdominal Girth (Maximum Pseudo-distension Combined with Maximum Lordosis)	F. Decrease in Circumference of Chest (Five Centimetres below Nipples) when A Changes to C
1	86·5	86·5	98·0	8·7	95·0	94·0 decreased to 91·5
2	77·5	77·5	86·5	8·1	84·0	91·5 decreased to 87·5
3	89·0	89·0	98·0	7·0	96·5	96·0 decreased to 94·5
4	89·0	87·5	96·5	9·2	93·5	101·5 decreased to 99·0

[1] All readings in centimetres. All measurements taken at end of quiet expiration.

pseudo-distension by voluntarily contracting their diaphragms, causing a mean descent of each diaphragmatic dome of 7·0 to 9·2 centimetres (measurement of radiographs at the end of quiet expiration).

In each control subject the abdominal pseudo-distension could be produced with or without associated exaggeration of lumbar lordosis. When lumbar lordosis was exaggerated without forcing down the diaphragm, there was no increase of abdominal girth, i.e. no pseudo-distension, though exaggerated lordosis increased slightly the convexity of the anterior profile of the abdomen.

In each subject the increase of abdominal girth attainable by voluntarily forcing down the diaphragm was actually reduced by exaggeration of lumbar lordosis (see Figures VIIIA, B, C and D, IXA and B, and Table II).

Each subject could breathe satisfactorily, though with effort and with irregularity of depth (cf. Case II), for a few minutes while maintaining the abdominal pseudo-distension; each found that maintenance of the pseudo-distension for more than a minute or two caused abdominal discomfort and, if persisted in, continuous abdominal pain, especially in the epigastric and lower substernal regions. Two

FIGURE VIIIA

Control subject I, a medical graduate, who could produce pseudo-distension of the abdomen at will. (All photographs taken at end of a quiet expiration.) Resting, no exaggerated lordosis, abdomen soft to palpation. Abdominal girth, 86·5 centimetres. Chest circumference five centimetres below the nipples, 94 centimetres

FIGURE VIIIB

Control subject I.—Voluntarily exaggerated lordosis allowing light to shine through from other side of subject. Anterior profile of abdomen appears slightly more convex; anterior abdominal wall slightly more taut. Abdominal girth unchanged at 86·5 centimetres

of the subjects later observed that the abdominal pain persisted in lesser degree for several hours after relaxing the abdominal pseudo-distension.

Intra-abdominal pressures were recorded in two of the control subjects as in Case II. By forcibly contracting the diaphragm to produce abdominal pressure increase of 45 to 60 centimetres of water for a few minutes, pressures during inspiration and expiration being recorded for 50 successive respiratory cycles; this caused considerable abdominal discomfort and some dyspnœa. Assumption of an exaggerated

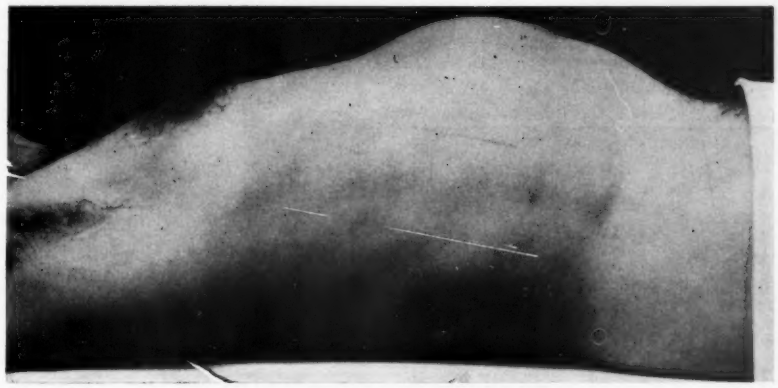

FIGURE VIIIc

Control subject I.—Voluntary pseudo-distension of the abdomen, without exaggeration of lumbar lordosis. Abdominal wall taut and firm to palpation. Abdominal girth increased to 98 centimetres. Note that the antero-posterior diameter of the chest is less than that of Figure VIIIA. Chest circumference five centimetres below the nipples, 91·5 centimetres

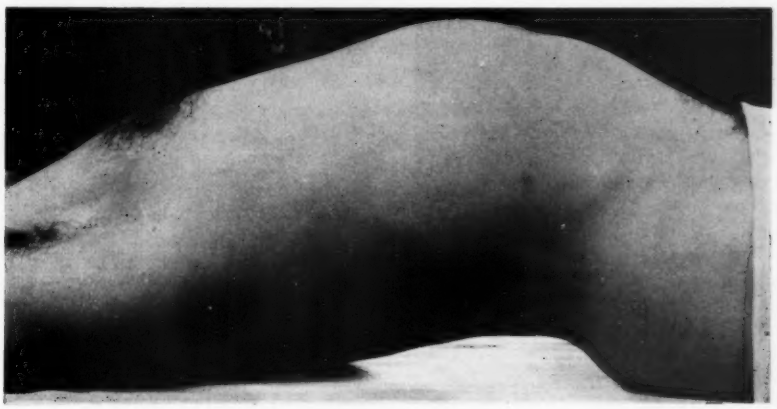

FIGURE VIIId

Control subject I.—Voluntary pseudo-distension of the abdomen with concomitant exaggeration of lumbar lordosis. Subject was unable to increase the abdominal girth quite as much as in Figure VIIIc, the girth measuring 95 centimetres

abdominal pseudo-distension, increases of intra-abdominal pressure of 60 to 70 centimetres of water above resting conditions were readily achieved, whether exaggerated lordosis was present or absent. Each subject, after practice, could breathe while maintaining an intra-lordotic posture without forcibly contracting the diaphragm had little effect on intra-abdominal pressure, the mean pressure for 50 successive respirations falling about one centimetre of water in one subject, rising about one and a half centimetres of water in the other.

Synchronously with the forced descent of the diaphragm to produce the abdominal pseudo-distension, the lower part of the thorax decreased slightly in circumference in each control subject, by an amount varying from 1·5 to 4·0 centimetres (see Figures VIIIA and VIIIC and Table II).

Passive flexion of the hips was found to hinder mechanically but not to prevent the maintenance of abdominal pseudo-distension in the consciously striving control subject.

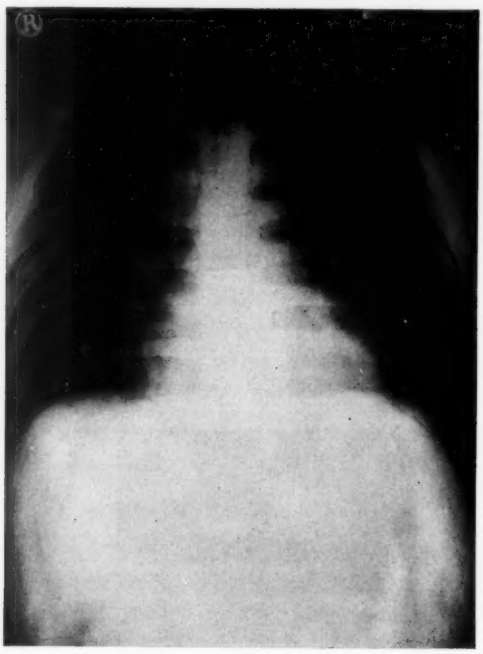

FIGURE IXA

Control subject I.—Radiograph of chest at rest (subject supine, end of a quiet expiration)

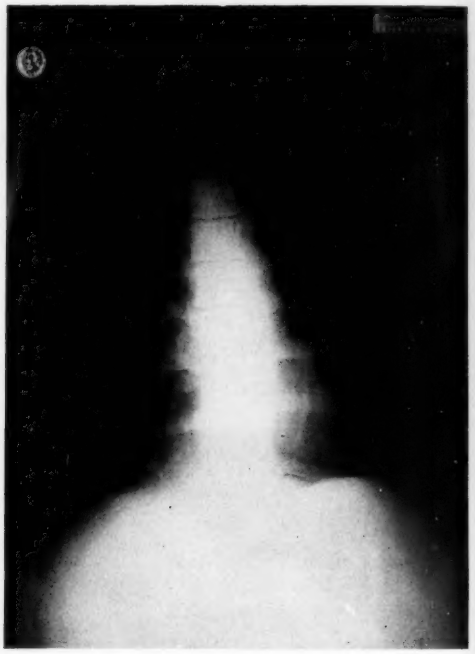

FIGURE IXB

Control subject I.—Radiograph of chest during stage of voluntary pseudo-distension of the abdomen; the domes of the diaphragm have descended a mean distance of 8·7 centimetres (subject supine, end of a quiet expiration)

DISCUSSION

Mechanism of Hysterical Pseudo-Distension of The Abdomen

Modern authors agree that there is no excess of gas in the digestive tract in hysterical pseudo-distension of the abdomen (Alvarez, 1949; Roussak, 1951; Westdahl, 1951; Stewart and McConnell, 1953; Ewing and Millar, 1955).

Alvarez (1949) gave a comprehensive review of earlier literature on the subject, citing authors who regarded exaggeration of lumbar lordosis as the most important factor, and others who were more impressed by descent of the diaphragm. His own opinion was that "hysterical abdominal bloating" was due "apparently to a contraction of the muscles lining the back and the upper end of the abdominal cavity. Sometimes, in addition, there may be a relaxation of the muscles of the anterior abdominal wall. These changes, associated often with the assumption of an extremely lordotic posture by the patient, tend to throw the abdominal contents forward and somewhat downward, toward the brim of the pelvis" (Alvarez, 1949). This view was accepted by Westdahl (1951) and by Stewart and McConnell (1953). Roussak (1951) stated that "these patients produce their abdominal distension by contraction of one or more portions of the musculature of the walls and roof of the abdominal cavity, with perhaps relaxation of the anterior and lateral muscles. Depression of the diaphragm, and lordosis maintained by sacrospinalis spasm, operate either singly or in conjunction, but the former appears the more important". Ewing and Millar (1955) concluded that the "abdominal protrusion is due, no doubt, to contraction of certain groups of

muscles of the abdominal wall and to relaxation of others. This is often accompanied by the development of a lumbar lordosis and by descent of the diaphragm ".

The study here presented attempts to analyse further the relative importance of exaggerated lumbar lordosis and descent of diaphragm in the production of hysterical abdominal pseudo-distension.

In Case I, the abdominal pseudo-distension was produced without any exaggeration of lumbar lordosis, while in Case II the increase of lumbar lordosis was trivial. In both these hysterics, when pseudo-distension was present, an impressive rise of the mean level of the domes of the diaphragm followed induction of light general anæsthesia (7·4 and 7·9 centimetres respectively). This seemed highly significant compared with the relatively slight alterations of diaphragmatic level (less than one centimetre in amplitude and inconstant in direction) when control subjects without abdominal pseudo-distension were given light general anæsthesia. As the investigations on the abdomens of the subjects in Cases I and II and on the control subjects were all carried out in the supine position, gravitational factors were clearly not involved in causing the abdominal changes. Further, in the stage of hysterical abdominal distension in Cases I and II the abdominal girth was the same in the erect as in the supine position. In both Case I and Case II sustained contraction of the diaphragm was regarded as the cause of the hysterical abdominal pseudo-distension.

The study of four apparently normal adult males who could produce abdominal pseudo-distension of comparable degree to that in Cases I and II indicated, however, that exaggerated lumbar lordosis by itself produces no increase of abdominal girth, i.e., no abdominal pseudo-distension. This is not to deny that exaggeration of lumbar lordosis may occur in some cases of hysterical abdominal pseudo-distension; it undoubtedly does occur (Alvarez, 1949; Roussak, 1951; Westdahl, 1951; Stewart and McConnell, 1953; Ewing and Millar, 1955). It is emphasized that exaggeration of lumbar lordosis by itself causes no increase of abdominal girth. It was of interest that on a number of occasions the voluntary forcing down of the diaphragm in the four control subjects tended to be automatically accompanied by exaggeration of lumbar lordosis; this tendency could readily be consciously inhibited.

In all four control subjects concomitant exaggeration of lumbar lordosis lessened the increase of abdominal girth that could be achieved by voluntarily forcing down the diaphragm. As exaggeration of lordosis itself produces some stretching of the anterior abdominal wall, it seems reasonable to infer that this limits the further stretching of the anterior abdominal wall attainable by forcing down the diaphragm.

Attention is drawn to the decrease in the horizontal dimensions of the lower part of the thoracic cage during the period of abdominal pseudo-distension in Case II and in each of the four control subjects when producing abdominal pseudo-distension. One of Roussak's illustrations of a hysterical patient shows this also, though Roussak (1951) does not comment specifically on the fact. This contraction of the lower part of the thoracic cage may possibly be passive in nature, due to the strong, sustained contraction of the diaphragm where it is attached to the thoracic cage, as the contraction of the lower part of the thoracic cage in the four control subjects appeared and disappeared synchronously with the voluntary strong contraction of the diaphragm.

When the intra-abdominal pressure was recorded during an episode of abdominal pseudo-distension in Case II, a pronounced rise of pressure was found (averaging approximately 59 centimetres of water). Roussak (1951) inferred a rise of intra-abdominal pressure in one of his patients with " hysterical abdominal proptosis ". The two control subjects whose intra-abdominal pressures were recorded showed comparable rises of pressure to that in Case II on production of abdominal pseudo-distension, and could breathe under these conditions though with some dyspnœa (see below).

Passive flexion of the hips sometimes abolishes hysterical pseudo-distension (Alvarez, 1949; Westdahl, 1951; Stewart and McConnell, 1953). This manoeuvre made more difficult though it did not prevent maintenance of abdominal pseudo-distension in the four consciously striving control subjects; it did not dissipate the hysterical pseudo-distension of the abdomen in Case II.

Terminology

The terms " hysterical meteorism " (Purves–Stewart, 1945), " hysterical, non-gaseous abdominal bloating " (Alvarez, 1949), " hysterical abdominal proptosis " (Roussak, 1951; Ewing and Millar, 1955), " hysterical abdominal distension " (Westdahl, 1951), " non-gaseous abdominal distention " (Stewart and McConnell, 1953) have been used in the literature more or less synonymously.

" Hysterical meteorism " is an incorrect term for there is no excess of gas within the abdomen.

" Hysterical abdominal bloating " and " hysterical abdominal distension ", connoting as they do an increase in volume of the abdominal cavity, are not entirely appropriate terms. The total volume of the abdominal cavity between diaphragm and pelvic floor is not increased but actually decreased, although the portion of abdominal wall between ribs and pelvis protrudes. Our finding that the intra-abdominal pressure is increased during the hysterical episode is in agreement with the inference of Roussak (1951). Hence, as the volume of any mass of gas is inversely proportional to the pressure, the temperature remaining constant (Boyle's Law), the gaseous abdominal contents will be reduced in volume; the volume of blood in low-pressure vessels such as abdominal veins and venules is also likely to be decreased if the intra-abdominal pressure rises significantly. Figure IIA in comparison with Figure IIB illustrates how, with the descent of the diaphragm, the vertical dimensions of the abdomen decrease during the hysterical phase, so that the total abdominal volume could be reduced despite an increase in the horizontal circumference of the abdomen between ribs and pelvis. Likewise, Figure IVA in comparison with Figure IVB illustrates increase in the vertical dimensions of the thoracic cavity during the hysterical episode. Thus there is only a local distension of the abdominal cavity between ribs and pelvis, but not a generalized abdominal distension.

Regarding the term " hysterical abdominal proptosis ", it may be noted that exaggeration of lumbar lordosis and thrusting forward of the abdomen and its contents produce no increase of abdominal girth; also, sustained strong contraction of the diaphragm forcing the abdominal contents caudally does not really represent a " falling down " of the abdominal contents, especially with the patient in the supine position or with the buttocks raised slightly above the level of the chest, in which postures abdominal pseudo-distension still readily occurs.

The term " hysterical pseudo-distension of the abdomen " is not open to the above objections. Prefixing " pseudo-" to " distension " connotes the spurious character of the apparent abdominal distension (i.e. the increase of abdominal girth); it is relevant that an external examination may take little account of the substantial decrease in the volume of those abdominal contents which are under cover of the lower thoracic cage. " Pseudo-distension " draws attention to the most important condition simulated, i.e., generalized distension of the abdominal cavity. This nomenclature is comparable to the term " pseudocyesis ", long used in obstetrics. The abdominal changes of this condition are closely related to hysterical pseudo-distension of the abdomen, though in addition there may be amenorrhœa, breast changes and other signs (Schopbach *et alii*, 1952).

Pain, Dyspnœa and Dysphagia in Relation to Pseudo-Distension of the Abdomen

It seemed significant that each of the four control subjects complained of abdominal discomfort mounting to severe continuous pain, especially in the epigastric and lower substernal region, if the abdominal pseudo-distension was maintained for more than a few minutes. Abdominal pain was a prominent complaint in both cases of hysterical pseudo-distension of the abdomen here described. Wolf (1947) described sustained contraction of the diaphragm as a cause of precordial pain in anxious persons.

It further seemed significant that maintenance of sustained abdominal pseudo-distension caused the four control subjects some difficulty in maintaining an adequate respiratory minute volume, i.e., dyspnœa was experienced. In Case II the subject complained spontaneously of " difficulty in getting his breath " in the stage of abdominal pseudo-distension. Wolf (1947) also drew attention to sustained contraction of the diaphragm as the mechanism of a common type of dyspnœa in anxious persons.

The dysphagia of which the subject in Case II complained when in the phase of abdominal pseudo-distension appeared to have an underlying objective reality, in that radiographic studies disclosed retention of opaque medium in the lower part of the œsophagus. This retention of opaque medium did not appear to represent a so-called " normal œsophageal ampulla " (Palmer, 1957), for each of the 12 control radiographs showed ready passage of the opaque medium into the stomach without any such " œsophageal ampulla ". (Six control radiographs were taken at 30-second intervals from the taking of the first mouthful of opaque medium on one day and repeated one week later.) The 12 comparable radiographs taken during two episodes of hysterical pseudo-distension of the abdomen all showed retention of opaque medium in the lower part of the œsophagus. These findings illustrate the contention of Wolf (1947), who described " occlusion of the cardiac end of the œsophagus during diaphragmatic spasm " in certain

anxious individuals who complained of difficulty in swallowing.

It has long been debated whether or not the right crus of the diaphragm acts as a pinchcock on the lower part of the œsophagus, causing constriction when the diaphragm descends during inspiration. Ingelfinger (1956) reviewed evidence for and against this view. In Case II the sustained contraction of the diaphragm with associated dysphagia and undue retention of opaque medium in the lower part of the œsophagus cannot be regarded as evidence of a pinchcock action of the diaphragm, because of the substantial rise of intra-abdominal pressure.

Negus (1943) drew attention to the importance of the different pressures to which the thoracic and abdominal segments of the œsophagus are subjected normally, a negative pressure in the former tending to open the thoracic part of the œsophagus, a positive pressure in the latter tending to close the abdominal part of the œsophagus. Negus emphasized the fact that the pressure difference is greater in inspiration and that this may halt a bolus of food, which enters the stomach when expiration commences.

Figure VII illustrates diagrammatically how a purely physical principle might account for delayed passage of opaque medium from the region of low intrathoracic pressure to the region of much-elevated intra-abdominal pressure (approximately 44 millimetres of mercury) in Case II during the stage of abdominal pseudo-distension. The range of phasic intra-œsophageal pressures varies from 20 to 50 millimetres of mercury according to Kramer *et alii* (1951) or from 20 to 100 millimetres of mercury according to Ingelfinger *et alii* (1954). Thus in Case II some œsophageal peristaltic waves might exert a pressure greater than the intra-abdominal pressure in the stage of pseudo-distension, and some might not. However Ingelfinger *et alii* (1954) cite evidence that œsophageal peristalsis ends several centimetres above the gastro-œsophageal junction, in which case it is not surprising that opaque medium was retained in the lower part of the œsophagus during the stage of abdominal pseudo-distension in Case II. Hiatus hernia, during the stage of abdominal pseudo-distension when the intra-abdominal pressure was raised, was excluded.

ACKNOWLEDGEMENTS

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EFFECTIVE TREATMENT OF A CASE OF METASTATIC THYROID CARCINOMA BY I^{131} AND DESICCATED THYROID[1]

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SUMMARY

The administration of desiccated thyroid inhibited the uptake, but not the release, of I^{131} (in five millicurie doses) by the metastases of a thyroid adenocarcinoma.

The desiccated thyroid was apparently responsible for a marked and sustained reduction in the size of the pulmonary and subcutaneous metastases.

It is suggested that in cases of thyroid carcinoma it may be better to try treatment with desiccated thyroid before I^{131} therapy, rather than after it.

WE report the effective treatment, first by I^{131} and later by desiccated thyroid, of a patient suffering from adenocarcinoma of the thyroid with multiple pulmonary metastases.

CASE HISTORY

A female child developed a goitre at the age of five years. Neither her brother nor her two sisters had a goitre. From the age of six months until the age of two and a half years, for cosmetic reasons, she had had several therapeutic doses of X-rays directed at birthmarks, one of which was situated at the back of her neck.

The goitre grew bigger, and surgery was advised when the child was seven years old. A pre-operative chest X-ray examination revealed multiple small shadows in the lung fields (Figure I). Thyroid carcinoma, with multiple pulmonary metastases, was diagnosed.

At this time (June 11, 1951), before any treatment, the thyroid was several times larger than normal size, and had become somewhat rectangular in outline (Table I). There were no palpable nodules, and the gland was wooden-hard, but was not fixed to the overlying skin. There were no enlarged lymph nodes palpable anywhere. The child was subjectively well. After administration of one millicurie of I^{131} to the child, there was a high counting rate over the normal thyroid area, but a low counting rate over the periphery of the mass in the neck, and no demonstrable I^{131} concentration in the thorax.

Sir Gordon Bell performed a thyroidectomy on June 12, 1951, as much as possible of the tumour in the neck being excised. In addition,

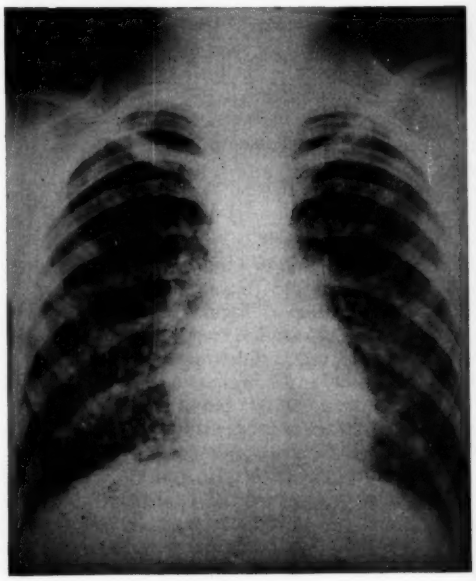

FIGURE I

Chest X-ray film of patient on June 7, 1951, showing the pulmonary metastases of the thyroid carcinoma before treatment

with a view to subsequent I^{131} therapy, all the normal thyroid tissue was excised.

Sections of the tumour showed it to be an adenocarcinoma, with areas of greater and lesser

[1] Received on March 21, 1958.

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differentiation. Where obvious acini were present, they contained colloid. Invasion of the blood vessels had occurred. Autoradiography showed patchy I^{131} uptake by some parts of the well-differentiated portions of the tumour, but none in the more anaplastic parts. Because of this demonstration of uptake of I^{131} by parts of the tumour, we decided to embark upon I^{131} therapy.

Methods

The uptake and release of I^{131} from the tumour metastases was followed by both total body counting and urine counting, the two methods giving results in good agreement.

Total Body Counting.—The total radioactive content of the patient was measured by seating her on a high stool, 12 feet from an unshielded Geiger tube (G. 10 Pb., 20th Century Electronics, London) placed horizontally three feet above the floor. The geometry was kept constant, enabling repeated comparable measurements to be made, and was calibrated by counting the patient immediately after she had drunk a known amount of I^{131}. No significant trend in the counting rate was observed in the following two hours as the I^{131} was redistributed in the patient's body.

Urine Counting.—This was done as described previously (Adams and Purves, 1956).

Distribution Counting.—The distribution of radioactivity in the patient's body, including the subcutaneous metastases, was determined

TABLE I

The Effect of I^{131} Therapy and of Thyroid Siccum on Patient Suffering from Thyroid Carcinoma with Pulmonary Metastases

Date	Neck	Chest X-Ray	Treatment	%age dose of I.131 in tumour	½ stay of I^{131} in Tumour.	Comment
11.6.51		Multiple shadows				
12.6.51			Excision of thyroid and primary tumour	I^{131} conc. in parts of tumour revealed by radioautography		
15.7.51		Multiple shadows	38 mC I^{131}	58% at 24 hours		Good I^{131} uptake by pulmonary metastases.
19.9.51		Multiple shadows	60 mC I^{131}	56% at 3 days		Appearance of nodules in neck, despite previous 38 mC I.131.
18.4.52		Multiple shadows	50 mC I^{131}	44% at 3 days	7.5 days	I^{131} uptake reduced. Disappearance of 2 neck lumps, appearance of another.
14.11.52		Partially cleared	5 mC I^{131}	40% at 3 days	9.5 days	Release rate slower than with 50 mC dose. Chest shows some clearing.
23.11.52 until 18.1.53			Thyroid siccum ½ to 3 gr. daily			I^{131} uptake markedly reduced after 2 months on desiccated thyroid.
12.1.53			5mC I^{131}	2.5% at 3 days		
11.3.53		Unchanged	5 mC I^{131}	38.5% at 3 days	9.4 days	Uptake has returned after cessation of desiccated thyroid.
14.5.53 until 29.5.53			Thyroid siccum 3 gr. daily			Desiccated thyroid started one day before I.131 given, has affected neither uptake nor release. A new lump has appeared in neck.
15.5.53		Unchanged	5 mC I^{131}	34% at 3 days	9.2 days	
22.10.53		Unchanged	30 mC I^{131}	28% at 3 days	7.2 days	I^{131} uptake has been reduced by the 5 mC doses.
5.11.53 onwards			Thyroid siccum 3 gr. daily			Complete clearance of chest X-ray and marked shrinkage of neck lumps after 22 months on desiccated thyroid.
31.8.55		Completely clear	Thyroid siccum 3 gr. daily			
29.8.56		Completely clear	Thyroid siccum 3 gr. daily			No change after a further year on desiccated thyroid.

by making counts with a variety of directionally-shielded Geiger tubes of varying sensitivity.

The Effect of Therapeutic Doses of I^{131} *on the Size and Iodine Metabolism of the Tumour Metastases*

From the table it is seen that the second therapeutic dose of I^{131} (60 millicuries) was followed by the disappearance of two lumps which had appeared in the neck after the first therapeutic dose. The third therapeutic dose (50 millicuries) failed to affect the size of another

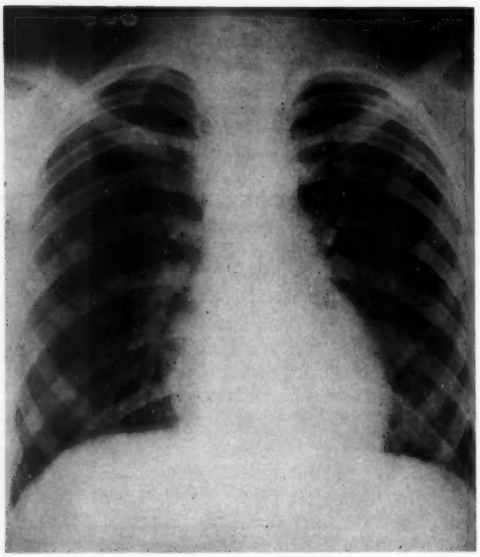

FIGURE II

Chest X-ray film of patient on August 31, 1955. After administration of 198 millicuries of I^{131} and two years' treatment with desiccated thyroid, there are no visible metastases

lump which had appeared in the neck. After this dose, however, the lung fields showed a partial clearing.

The table also shows that several doses of five millicuries failed to affect either the lump remaining in the neck or another one which appeared later. No further clearing of the lung fields occurred during the period of administration of the five millicurie doses.

It can be seen from the table that the five millicurie doses were as effective as the larger doses in reducing the I^{131} uptake of the tumour. It is also seen that the rate of release of I^{131} from the tumour was much greater than that from a normal thyroid gland, in accordance with the observations of Pochin *et alii* (1954). The five millicurie doses were released from the tumour slightly more slowly than were the larger doses.

The Effect of Desiccated Thyroid on the Iodine Metabolism and Size of the Tumour Metastases

From the table it is seen that desiccated thyroid, exhibited over the preceding two months, almost completely inhibited the uptake of I^{131} by the tumour metastases. However, it is seen that desiccated thyroid had no effect on the rate of release from the tumour of a five millicurie dose of I^{131}, when exhibition of the desiccated thyroid was commenced one day before administration of the I^{131}.

It is seen from the table that after exhibition of desiccated thyroid for two years, chest X-ray examination showed complete clearing of the lung fields (Figure II). In addition, the two lumps in the neck had shrunk much, becoming only one to two millimetres in diameter. On August 29, 1956, after a further year of treatment with desiccated thyroid, the picture was unchanged.

A few months later the lumps in the neck enlarged, but promptly shrank again when the dose of desiccated thyroid was increased to four grains daily. The girl now weighed eight stone ten pounds, and was nearly mature sexually.

A chest X-ray examination was made on 21st May, 1957, when the lung fields were found to be still clear. The clinical picture was unchanged when the girl was examined in June, 1958.

DISCUSSION

The Optimum Size of Therapeutic Doses of I^{131}

The autoradiographs showed that the uptake of I^{131} by the tumour was p .tchy. Subsequently it appeared that when the functioning portion of the tumour was destroyed by I^{131}, another portion developed thyroid function, this being repeated many times. In such circumstances, a given amount of I^{131} will be more effective if subdivided into several doses, rather than given all at once. This reasoning led to our decision to try the effect of five millicurie doses.

As already described, the five millicurie doses were as effective as the larger doses (38 to 60 millicuries) in reducing the I^{131} uptake of the tumour. This indicates that the five millicurie doses were as effective as the larger doses in destroying the iodine-concentrating portions of the tumour. The slightly slower release rate of the five millicurie doses (half-stays 9·5, 9·4, 9·2 days) showed that the larger doses (half-stays 7·5, 7·2 days) achieved the destructive effect faster, as would be expected.

Distribution counting showed that the concentration of I^{131} in the lumps in the neck was falling with successive therapeutic doses, but the failure of these lumps to decrease in size showed that they were composed mostly of non-iodine-concentrating tissue, not susceptible to I^{131} therapy. Therefore, there appeared to be no prospect of eradicating the tumour with I^{131}.

With this in mind, we terminated the I^{131} therapy with a final dose of 30 millicuries, and commenced exhibition of desiccated thyroid.

The Therapeutic Effect of Desiccated Thyroid

It is possible that the final complete clearing of the patient's chest and the shrinking of the lumps in the neck were due to the last therapeutic dose of I^{131} (see Table I). In the interest of the patient, once it had been decided that desiccated thyroid might be of therapeutic value, we felt it unjustifiable to withhold this medication until the effect of the last I^{131} dose could be assessed. However, a total dosage of 163 millicuries of I^{131} had produced only a moderate therapeutic effect, and it appears improbable that a further 30 millicurie dose should be strikingly effective. Moreover, one of the lumps in the neck had already been quite unaffected by a dose of 50 millicuries of I^{131}, as well as three doses of five millicuries.

It was the observation that metastasizing thyroid tumours in rats are sometimes thyrotrophin-dependent (Purves and Griesbach, 1947; Purves, *et alii*, 1951) which prompted us to try the effect of thyrotrophin-deprivation on this tumour. Recently, four other groups of workers have reported a therapeutic effect from administering thyroid hormone to patients with thyroid carcinoma (Balme, 1954; Crile, 1955; Maloof *et alii*, 1956; Thomas, 1957).

The finding that some metastasizing thyroid carcinomata are thyrotrophin-dependent is analogous to the demonstration of the hormone dependence of certain breast and prostate carcinomata. The administration of desiccated thyroid achieves a specific hypophysectomy, in preventing pituitary secretion of thyrotrophin.

General Considerations

The most malignant types of human thyroid carcinomata do not concentrate I^{131} and, if we may judge from experience with rat tumours, will be unaffected by deprivation of thyrotrophin. By natural selection tumours tend to become more malignant, faster growing mutants overshadowing slower growing ones. It appears that mutations occur frequently in tumour cells. Thus, a carcinoma which is thyrotrophin-dependent may at any time produce an autonomously growing variant. Irradiation is known to increase the frequency of occurrence of mutations. Therefore I^{131} therapy probably increases the likelihood of the occurrence of an autonomous variant of a thyrotrophin-dependent tumour. For this reason, as the possibility of eradicating a thyroid carcinoma by I^{131} therapy is remote, it may be that treatment with desiccated thyroid should be tried before I^{131} therapy is attempted.

Whilst it is unlikely that a high percentage of cases of thyroid carcinoma will respond to desiccated thyroid, it is possible that the figure may be better than that for response to I^{131} therapy. Both these therapeutic measures are most likely to be effective in the better-differentiated thyroid carcinomata, and it may be that the tumours which respond to I^{131} are also the ones which would respond to desiccated thyroid, and vice versa.

Should desiccated thyroid prove to be as effective as I^{131}, then the former would be the therapeutic agent of choice, as its use does not entail radiation exposure.

ACKNOWLEDGEMENT

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THE EFFECT OF UNILATERAL RENAL ISCHÆMIA ON FLUID TURNOVER IN RATS[1]

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SUMMARY

Modifications in fluid handling occur in rats following the induction of unilateral renal ischæmia, even though the other kidney remains intact. Some animals develop extreme degrees of polydipsia and polyuria. Amongst those drinking 0·5% saline, "polydipsia", defined as an intake more than three times the original, was observed during an observation period of four weeks in 16%. Amongst animals drinking water, this degree of increase in fluid turnover was observed in only 4%.

Apart from these animals, spontaneous fluid turnover was not demonstrably greater than that which could have been accounted for by diminution in functioning renal tissue consequent upon the induction of ischæmia. Alterations in fluid handling in these animals attributable to the hypertension are, however, demonstrable by measurement of the rate of rejection of an orally administered fluid load. Following the induction of ischæmia, rejection of both saline and water was greater than either before this procedure or after removal of the ischæmic kidney, and the degree of increase in both groups was the same.

Histological examination of the kidneys showed a close correlation between the onset of polydipsia and polyuria and the development of malignant hypertensive changes in the intact kidney.

Markedly increased fluid turnover apparently indicating the supervention of the malignant phase is described in a patient with unilateral renal disease, and the literature on this type of case is briefly reviewed.

IT has been repeatedly demonstrated that modifications in various aspects of fluid and electrolyte metabolism occur in hypertension, both clinical and experimental. Amongst the manifestations of altered fluid handling which have been described is an increased rate of fluid turnover, either spontaneous or in response to load. Thus polydipsia and polyuria have been demonstrated in experimental hypertension in the rat resulting from renal ischæmia, even when unilateral, the other kidney remaining intact (Oster and Martinez, 1943; Braun-Menendez, 1954). It has been reported also in human hypertension resulting from unilateral renal disease (Deming, 1954; Snyder *et alii*, 1955). In the cases reported by these and other authors (*vide infra*) the grossly increased fluid turnover was arrested by nephrectomy in spite of the consequent reduction of total renal tissue, indicating that it did not have its basis in renal insufficiency.

Additional evidence of altered fluid and electrolyte handling in hypertension has been provided by experiments in which the rate of rejection of a fluid load has been measured. An increased rate of rejection has been demonstrated in human hypertensives by Birchall *et alii* (1953), Green *et alii* (1954), Ek (1955), Hollander and Judson (1957) and others, and in hypertensive rats by Friedman *et alii* (1955). These rats had been made hypertensive by induction of ischæmia in solitary kidneys, unilateral nephrectomy having been carried out as a preliminary.

In the present experiments further observations have been made on the effect of unilateral renal ischæmia on fluid turnover in rats, the other kidney, however, being left intact.

The gross increases in fluid turnover which occurred in a number of our animals were found on histological examination of the kidneys to be associated with the development of malignant hypertension.

Some observations are also reported on a patient with unilateral renal disease who developed malignant hypertension and increased fluid turnover.

EXPERIMENTAL METHODS

Male white Wistar rats aged from four to six months were used throughout. They were kept in individual cages with drinking bottles so arranged as to minimize accidental loss. The

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animals were given 0·5% saline or water to drink. What was being measured therefore was the self-selected intake of 0·5% saline or water. Urine volumes were measured in a number of animals and were observed to correspond to intake. The food consisted of compressed pellets of grain and meat meal which contained approximately 1·15 grammes of sodium per 100 grammes of pellets.

Fluid rejection experiments were carried out by administering to each rat by gavage a quantity of water or saline as specified later. The rats were then placed in collecting funnels over measuring cylinders in which the volumes of urine passed at specific intervals after gavage were recorded.

The rats were allowed free access to their drinking water until the time of administration of the gastric load. Hypertension was induced by the method of Wilson and Byrom (1939), a silver clip with a gap of 0·095 of an inch being applied to the renal artery of the left kidney.

Blood pressures were recorded under light ether anæthesia using the techniques described by Gallagher and Grimwood (1953).

The animals in the present series of experiments were kept under observation for a period of four weeks from the time of application of the constricting clip. Histological examination of clipped and intact kidneys was later carried out in a number of the animals. Sections were stained with van Gieson's stain and with hæmatoxylin and eosin.

RESULTS

Fluid Turnover

(*a*) *Animals Drinking* 0·5% *Saline.*—After the induction of ischæmia in one kidney there was a generalized increase in fluid turnover. The increase was of small although significant degree in most animals, but in about one in six extreme degrees of polydipsia and polyuria developed at some time during the period of observation. The maximum post-clipping saline intakes, each in terms of its own pre-clipping average intake, are shown in Table I. Here they are grouped in whole number multiples of the average pre-clipping intake for each.

If polydipsia is defined arbitrarily as a fluid intake more than three times their own average pre-clipping intake, 27 of 168 rats developed "polydipsia" (16·1%). The highest daily intake by any one rat was 348 millilitres. This rat weighed 220 grammes. A corresponding intake for a 70-kilogramme man would be 111 litres, or about 200 pints a day. Such a comparison is not really justifiable, however, as a rat ordinarily drinks a relatively large amount compared with a human.

The grossly increased fluid turnover has usually appeared fairly suddenly and in most cases within the first three weeks after application of the constricting clip. The time of onset may be seen in Figure I. Polydipsia has become apparent for the first time as late as six weeks, although the animal concerned is not included in this series, and may well have been observed later in other instances had more animals been kept under observation for a prolonged period. The blood pressure at the time of onset of the polydipsia was frequently above the average at that time interval after clipping, although in some instances it was never observed to be particularly high. The rate of rise of blood pressure in the whole group is shown in Figure I, together with the last blood pressure reading before the onset of polydipsia.

TABLE I.

Highest Intake after Clip by Each Rat÷Average Intake by Same Rat before Clip

Ratio	Number of Rats	
<1·00	0	
1·00– 1·99	120	
2·00– 2·99	21	
3·00– 3·99	10	"Polydipsia"
4·00– 4·99	3	
5·00– 5·99	4	
6·00– 6·99	5	
7·00– 7·99	3	
8·00– 8·99	0	
9·00– 9·99	1	
10·00–10·99	0	
11·00 and over	1	

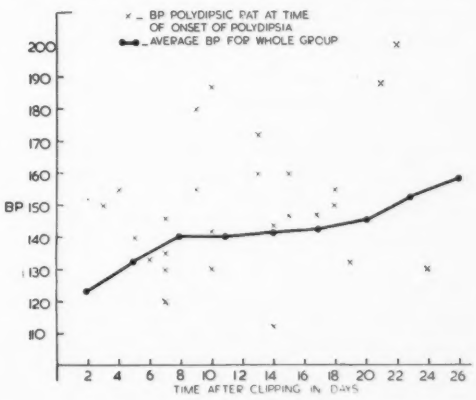

FIGURE I

Blood pressures at time of onset of polydipsia

The polydipsia and polyuria might last for three or four days and then pass off spon-

taneously, the fluid intake then returning to more usual levels (although generally somewhat above the average), or it might be sustained. Several temporary episodes might occur during the period under observation. The development of sustained polydipsia and polyuria was often associated with deterioration in the condition of the animals and a fall in blood pressure. They lost weight rapidly; some developed encephalopathic seizures, following which, however, there was often a sharp fall in fluid intake, or hæmaturia and/or melæna became evident. Untreated animals with sustained polydipsia usually died in a state similar to that described as "vascular crisis" by Wilson and Byrom (1939). With removal of the originally ischæmic kidney, polydipsia and polyuria were abolished, and the general condition of the rat improved. Amongst our animals with sustained polydipsia excision of the originally intact kidney has been rapidly followed by death. No animal has been observed to develop polydipsia after unilateral nephrectomy without clipping.

TABLE II.

Twenty-Four Hour Intake in Rats Drinking (a) 0·5% Saline, (b) Water before and after Induction of Unilateral Ischæmia and after Removal of Ischæmic Kidney, Rats with Polydipsia Excluded

Rats Drinking	Twenty-Four Hours Intake (Millilitres)		
	(i) Before Renal Ischæmia	(ii) During Renal Ischæmia	(iii) After Removal of Ischæmic Kidney
(*a*) 0·5% saline (24 rats)	25·4	33·1	35·5
(*b*) Water (24 rats)	22·2	24·5	25·2
In both (*a*) and (*b*)	(ii)–(i) Differences significant: $0·01 > P > 0·001$ (iii)–(ii) Differences not significant: $0·2 > P > 0·1$		

The characteristics of the state of polydipsia and polyuria will be discussed in detail in a subsequent paper.

There was, as remarked earlier, a smaller but still significant increase in fluid turnover observable even in those rats which did not develop "polydipsia" as previously defined. This could have been attributed to the diminution in functioning renal tissue consequent upon the application of a constricting clip to the renal artery.

In Table II is shown the effect of removal of one kidney after a period of ischæmia in a group of non-polydipsic animals. It can be seen that the fluid intake remained at a level (significantly) higher than the original intake and not significantly different from that during the period of ischæmia. Apart from the rats which developed the polydipsia-polyuria syndrome there is no evidence from these data to show that fluid turnover is increased except to the extent that might have resulted from diminution in functioning renal tissue.

(*b*) *Rats' Drinking Water.*—The daily intake of rats given water to drink was less than that of those receiving 0·5% saline, the average before operation being 22·2 millilitres per day (S.D. 2·2) and 27·6 millilitres per day (S.D. 4·7) respectively. After application of the constricting clip to one of the renal arteries gross increases in fluid intake were much less common than in the case of the animals on 0·5% saline. Two animals out of 48 developed polydipsia (4·2%).

This is of particular interest because the degree of blood pressure elevation after induction of unilateral renal ischæmia was not obviously less marked in the rats' drinking water than in those drinking 0·5% saline. Blood pressures before, four weeks after the application of the constricting clip, and two weeks after removal of the ischæmic kidney are shown in Table III.

TABLE III.

Blood Pressures (Mm. Hg)

Drinking Fluid	Blood Pressures (Mm. Hg)		
	Before Application of Clip	During Period of Ischæmia of One Kidney	After Removal of Ischæmic Kidney
Water	127 (S.D. 11)	157 (S.D. 11)	116 (S.D. 27)
0·5% saline ..	128 (S.D. 15)	160 (S.D. 17)	126 (S.D. 17)

Apart from the two animals developing polydipsia there was a small increase in the average daily turnover to 24·5 millilitres, the difference from the control level being significant ($·01 > P > ·001$). These results are tabulated in Table II from which it can be seen that, though the intake after application of the constricting clip was significantly greater than during the control period, it did not differ significantly from that following removal of the ischæmic kidney. There is thus no indication from the data afforded by these observations of any acceleration of fluid turnover induced by unilateral renal ischæmia in animals drinking water, other than that which could be attributed to diminution of total functioning renal tissue, except in the case of the two polydipsic animals.

Fluid Rejection After Load

An accelerated fluid turnover in the non-polydipsic animals during the period of hypertension was, however, shown by estimation of the rate of rejection of fluid load administered by gavage.

Volume of urine excreted within one and a half hours of administration of an intragastric fluid load were measured. The experience of Friedman *et alii* (1955) and our own preliminary experiments indicated that this includes the peak of the diuresis resulting from an intragastric load of this order.

Rejection was estimated in nine non-polydipsic rats drinking saline. The load consisted of nine millilitres of 1·5% saline given by gavage. The rats weighed between 250 and 340 grammes, with an average weight of 280 grammes. Four estimations were made on each rat before the induction of renal ischæmia, four after this, and four after removal of the ischæmic kidney, the observations being made twice weekly (Table IV).

TABLE IV.

Rejection of Fluid Load. Fluid Excreted Within One and a Half Hours of Oral Load

Load	Fluid Excreted Within One and a Half Hours of Oral Load		
	Before Application of Clip	During Period of Ischæmia of One Kidney	After Removal of Ischæmic Kidney
Saline load (mean of 9 rats)	3·11 (S.E. 0·28)	4·53 (S.E. 0·28)	3·09 (S.E. 0·28)
Water load (mean of 21 rats)	3·06 (S.E. 0·24)	4·15 (S.E. 0·15)	3·22 (S.E. 0·24)

Rejection was estimated in 21 non-polydipsic rats (weights 270 to 350 grammes, average 300 grammes) drinking water. The oral load was based on weight, each animal receiving three millilitres per 100 grammes. Two comparable estimations were made on each rat before and five after induction of renal ischæmia. Two further estimations were made after removal of the ischæmic kidney (Table IV).

In each case volumes rejected during the ischæmic kidney period were significantly greater than those either before induction of ischæmia or after removal of the ischæmic kidney ($P < \cdot001$). In neither case was there a significant difference between amounts rejected at estimations in the first and third periods.

It is of additional interest that there was no significant difference between the amounts excreted at corresponding estimations in the saline and water load experiments. The water load rejection experiments were actually carried out first and the amounts of fluid load varied according to the weights of the animals. Analysis of covariance showed that significant adjustments could be made to the mean urine returns, but that these adjustments accentuated significant differences and reduced non-significant ones.

In the saline load experiments the fluid load was the same for each rat. Analysis of covariance showed that no significant adjustments could be made to the urine returned on the basis of concomitant variation in the weight.

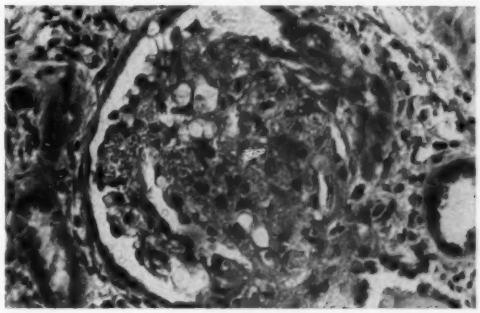

FIGURE II

"Coagulative" necrosis in glomerulus of originally intact kidney

The results of these observations indicate that the induction of renal ischæmia causes generalized increases in fluid turnover, which are, however, demonstrable in most cases only in terms of rejection of a fluid load. This increased rate of rejection appears to be of comparable degree with both saline and water loads.

Some animals develop extreme increases in fluid turnover, and this syndrome of polydipsia and polyuria appears to eventuate more frequently in animals given 0·5% saline as drinking fluid than in those drinking water.

Histological Changes

Histological examination of the kidneys was carried out in a number of these animals to see whether any differences were manifest between the rats with the polydipsia syndrome and those without.

The kidneys of 51 animals were examined, of which 15 had had polydipsia.

The kidneys to which the constricting clip had been applied, although apparently normal

TABLE V.

Relationship Between Incidence of Polydipsia and Histological Changes in Kidneys

Subjects	Highest Fluid Intake (Millilitres)			Clipped Kidney: Severity of Changes				"Intact" Kidney	
	Mean	Range	S.D.	None	Mild	Moderate	Severe	Glomerular Capillary Necrosis	Arteriolar Necrosis
15 polydipsic rats	157	91–348	68	0	3	3	9	15 of 15	12 of 15
36 rats without polydipsia	45	19–69	11	9	15	5	7	4 of 36	1 of 36

in some, showed in most cases a greater or lesser degree of ischæmic change. This varied from mild simple atrophy to gross ischæmia with areas of necrosis, cellular infiltration and severe contraction. The appearances in these kidneys have been classified as normal or showing mild, moderate or severe ischæmic change.

The most striking histological features were the appearances in the originally intact kidneys of the animals with the polydipsia–polyuria syndrome. These showed the changes

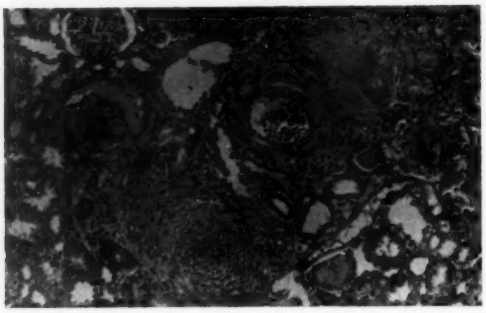

FIGURE III

Arteriolar necrosis in originally intact kidney

resembling those of malignant hypertension in man which were described by Wilson and Byrom (1939). Glomerular necrosis was present in all, and this took forms varying from patches of local necrosis or large areas of "coagulative" necrosis to hæmorrhagic infarction of the glomerulus. This change is illustrated in Figure II. In 13 of the 15 polydipsic animals, arteriolar necrosis was present, and it was of gross degree in most cases. This appearance is illustrated in Figure III. In only one of the 36 animals not showing the gross increase in fluid turnover were the changes of arteriolar necrosis present, and only in this animal and in three others was glomerular capillary necrosis evident, the change in these three being of mild degree. The incidence of histological changes in the clipped and intact kidneys are shown in Table V.

These findings suggest that the occurrence of the polydipsia–polyuria syndrome is indicative of the onset of the malignant phase of hypertension.

DISCUSSION

Polyuria has long been recognized as accompanying clinical states of chronic renal insufficiency and also as occurring experimentally in animals in which the amount of renal tissue has been drastically reduced (Bradford, 1899; Chanutin and Ferris, 1932). It has not been so widely appreciated that a greatly increased fluid turnover may occur with much smaller reductions of functioning renal tissue when this is associated with severe hypertension. The present experiments appear to indicate that the onset of gross polydipsia and polyuria in rats with hypertension resulting from unilateral renal ischæmia, the other kidney remaining intact, indicates the supervention of malignant hypertension in the originally intact kidney. Attention was drawn by Deming (1954) to the occurrence of similar marked increases in fluid turnover in human patients with severe hypertension resulting from unilateral renal disease. He reported a case in which polydipsia and polyuria apparently marked the development of malignant hypertension, resulting in his case from a vascular anomaly of the artery of the diseased kidney. Both increased fluid turnover and hypertension were relieved by nephrectomy. From a confessedly incomplete review of the literature he was able to quote nine cases in which severe hypertension resulting from unilateral renal disease appeared to be associated with polyuria.

A particularly striking example was that reported by Snyder *et alii* (1955). A child of nine months had extremely severe hypertension resulting from obstruction of an anomalous renal artery. Polydipsia (daily intake two to three litres) and polyuria were evident in hospital

prior to nephrectomy resulting in cure of the hypertension. Subsequently the fluid intake diminished to less than a litre daily. Maximum specific gravity attainable was 1·008 before operation and 1·030 after operation.

In the case reported by Marks (1955) severe hypertension developed in pregnancy and was accompanied by "frequency of micturition without scalding, which persisted until operation and became such that she passed urine six times by day and six times by night". After delivery the severity of the hypertensive state continued to increase until relieved by removal of a contracted chronic pyelonephritic kidney; after this the fluid turnover also returned to normal.

Hunter and McElmoyle (1956) reported a case of unilateral renal infarction with hypertension becoming malignant and causing encephalopathy, which was relieved by nephrectomy. Two days before the onset of encephalopathy the patient was reported to have had an abnormal craving for salt and to have been drinking great quantities of water.

Our attention was directed to this phenomenon by the case of a girl of 18 years (case no. 43), who had had moderately severe hypertension from the age of 12 years, which resulted from a congenital abnormality of the bladder producing bilateral hydroureter and hydronephrosis. Repeated episodes of infection of the renal tract had led to virtually complete loss of function of the left kidney. However, apart from the symptoms associated with urinary infections, she had felt relatively well till early in 1956, when she noticed nocturia for the first time, and found herself passing large quantities of pale urine with no dysuria. She felt also that her general health had deteriorated. On examination in May 1956, casual blood pressure readings of as high as 240/160 mm. Hg. were recorded, and the fundi showed the changes of malignant hypertension.

It is of interest that the polyuria was arrested on successful control of the blood pressure with ganglion-blocking drugs.

This phenomenon is obviously not an invariable or even a frequent concomitant of malignant hypertension, but it may be a highly significant one in drawing attention to the possibility of unilateral renal disease with hypertension in the accelerated phase.

Reverting to the animal experiments, it is of interest that, although the onset of the polydipsia–polyuria syndrome is clearly correlated with the vascular changes in the "intact" kidney, the degree of ischæmia in the polydipsic group was also on the average greater than in the non-polydipsic group. Since in several cases with malignant hypertensive changes in the "intact" kidney ischæmia of the clipped kidney was mild, and since *per contra* gross ischæmia in the clipped kidney occurred quite often without polydipsia, the degree of ischæmia in the clipped kidney is not a potent factor in the production of the syndrome. It may well be that the greater severity of these changes in the polydipsic group results from a somewhat greater tendency for severe clipping to give rise to a severe hypertensive state, although this correlation is not a close one as emphasized by Wilson and Byrom (1939).

These observations on fluid turnover indicate the dramatic manner in which fluid metabolism is modified in rats developing malignant hypertension. The fluid rejection experiments show that less striking but statistically highly significant alterations in fluid metabolism exist in the hypertensive animals which do not develop malignant vascular changes or polydipsia. Friedman *et alii* (1955) demonstrated that rats made hypertensive by the application of a figure-of-eight ligature to the sole remaining kidney had a more rapid rate of rejection of an administered fluid load than a series of comparable uninephrectomized controls. These experiments did not, however, exclude the possibility that reduction of functioning renal tissue consequent upon the application of the ligature might have been responsible for the increased rate of fluid turnover. The present experiments indicate that the altered state must be related to the hypertension itself and not to the reduction in functioning renal tissue resulting from the induction of ischæmia. After excision of the clipped kidney, with further reduction of renal tissue to the extent that this kidney remained functional, the rate of rejection nevertheless reverted to the same level as before induction of ischæmia.

The phenomenon of increased rate of rejection of a fluid load in hypertension has also been demonstrated in humans by a number of workers (Birchall *et alii*, 1953; Thompson *et alii*, 1954; Green *et alii*, 1954; Ek, 1955; Hollander and Judson, 1957). The identical degree to which rejection of both water and saline loads by non-polydipsic animals drinking water and saline respectively was increased during the hypertensive phase suggests that fluid handling in each group was modified to a basically similar degree. The higher incidence of polyuria in the group drinking saline was apparently due to the more frequent incidence of malignant vascular change in these animals. The higher incidence of secondary vascular lesions in hypertensive animals drinking saline has also been evident in other experiments.

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LEFT VENTRICULAR HYPERTROPHY: ELECTROCARDIOGRAPHIC DIAGNOSIS[1]

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SUMMARY

The specificity of diagnostic criteria previously proposed for the diagnosis of left ventricular hypertrophy have been analysed by correlation of the isolated ventricular weight and the twelve-lead electrocardiogram, and by consideration of commonly associated clinical conditions and of the pathological state of the myocardium in 83 adult hearts.

As an aid to the clinical diagnosis of left ventricular hypertrophy in adults over 30 years of age it is concluded of the electrocardiogram: (a) That lesser degrees of left ventricular hypertrophy cannot be accurately diagnosed by any criteria described to date; (b) That, where left ventricular weight exceeds normal by 40%, accurate positive diagnosis can be made in approximately half the cases, and that this proportion increases with the degree of hypertrophy; (c) That only the QRS voltage in the electrocardiogram has any direct relationship to left ventricular weight and is therefore of diagnostic value; (d) That the useful diagnostic criteria may be reduced to two or possibly three: the maximum $R+S$ voltage in the precordial "V" leads; maximal precordial $R>26$ millimetres; $R1+S3>25$ millimetres; (e) That the diagnostic application of relevant QRS voltage criteria is not materially restricted by the administration of digitalis or by the presence of congestive cardiac failure or lesser degrees of ischæmic heart disease. Advanced ischæmic disease involving extensive destruction of the myocardium may reduce QRS voltage and thence its diagnostic value.

The term "left ventricular strain" has little meaning, since the electrocardiographic patterns to which it is applied cannot be directly related to left ventricular hypertrophy or to any one physiological, biochemical or dynamic factor recognized at present.

CLEARLY it is improbable that the linear electrocardiogram will predict infallibly the presence and degree of left ventricular hypertrophy, but it is equally apparent that much of the existing diversity of opinion on the subject stems from uncritical observations. The left ventricular mass can be determined with accuracy only by isolation of the ventricles (Lewis, 1914; Hermann and Wilson, 1922; McPhie, 1957), but this basic measurement has rarely been applied to electrocardiographic studies.

Scott *et alii* (1955) assessed left ventricular hypertrophy from the ventricular thickness and total heart weight in very selected cases when they analysed the electrocardiographic diagnostic criteria proposed by other workers (Gubner and Ungerleider 1943; Wilson, *et alii*, 1944; Katz 1946; Noth, *et alii*, 1947; Goldberger 1949; Sokolow and Lyon 1949; Schach, *et alii*, 1950; Goulder and Kissane 1951). It is very significant that, despite the heavy bias towards positive findings, only the criteria of Sokolow and Lyon (1949) showed any useful correlation with total heart weight.

Gubner and Ungerleider simply studied the bipolar limb leads in hypertensive individuals during life, while neither Wilson and his associates nor Goldberger (1944, 1949, 1950) offered any pathological data in support of the criteria they proposed. Kaplan and Katz (1941) described patterns of "left ventricular strain", and neither in this nor in subsequent surveys (Katz *et alii*, 1942; Katz, 1946; Rosenman, *et alii*, 1950; Schach *et alii*, 1950) was any real attempt made to isolate the influence of left ventricular hypertrophy from that of other factors in the presence of "left ventricular strain". Noth *et alii* (1947) published no detailed data from their 84 cases in which total heart weight was ascertained.

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For their comprehensive study Sokolow and Lyon (1949) selected 200 cases in which there were abnormal electrocardiograms and a "cardiac disorder capable of producing increased strain on the left ventricle". Even a subsequent investigation (Grubschmidt and Sokolow, 1957) of false positive diagnosis by *QRS* voltage criteria relied upon clinical and radiological assessment of left ventricular enlargement, with which hypertrophy is not necessarily synonomous.

Goulder and Kissane (1951) relied upon similar indirect methods.

It is evident that the growth of current views on the electrocardiographic diagnosis of left ventricular hypertrophy has centred on the amplification of abnormalities of the electrocardiogram with too little regard for the accurate determination of the condition which they purport to indicate. Consequently, the specificity of many diagnostic signs described is open to serious question. With that in mind this investigation was designed to test the real clinical value of the electrocardiogram in the diagnosis of left ventricular hypertrophy. Since deductions must perforce be drawn from the empirical correlation of two measureable phenomena, left ventricular mass and the corresponding electrocardiogram, it would seem essential that these measurements be accurately defined, that analysis be both quantitative and qualitative and that due consideration be given to other conditions known to influence the electrocardiogram and to the limitations of selection of material.

MATERIAL

The study was based upon 102 adult hearts which were obtained at routine autopsies in a public hospital and which were employed in the analysis of methods of determining left ventricular hypertrophy (McPhie, 1957). The inherent limitations to random sampling were accentuated by the necessity for a relatively recent electrocardiogram to have been recorded. The age and sex distribution of the subjects in the series (approximating that of Grubschmidt and Sokolow (1957)) and the cause of death have been defined in the earlier publication (McPhie, 1957).

Analysis of the 36 cardiac deaths revealed that 19 were due to recent myocardial infarction, five to hypertensive cardiac failure, four to aortic stenosis, two to rheumatic mitral incompetence and six to multiple causes. Cases of primary right heart disease were deliberately excluded.

METHODS

The method of isolation of the ventricles has been described elsewhere (McPhie, 1957) in a study which confirmed the conclusion of Fulton and his colleagues (Fulton *et alii*, 1952) that in the absence of primary right ventricular hypertrophy total ventricular weight is a reliable indicator of left ventricular weight. The correlation study was therefore based on this measurement, and 250 grammes was accepted as the upper limit of normality for total ventricular weight. Ultimately the ventricles and coronary arteries were sectioned extensively and studied macroscopically.

No means is known of expressing the adequacy of the coronary circulation in terms of relative demand and a fine distinction cannot be drawn between the absolute insufficiency of obliterative coronary disease and the relative ischæmia of myocardial hypertrophy (Blumgart *et alii*, 1940). The present material was classified therefore according to readily defined clinical and pathological criteria.

An arbitrary clinico-pathological classification of six groups was designed to simplify analysis. This is set out below.

Group 1. Coronary arteries and myocardium macroscopically normal. No factors included in other groups. (Not necessarily "normal" controls.)

Group 2. Non-cardiac factors possibly influencing the electrocardiogram. (*a*) Digitalis, (*b*) Electrolytic disturbances, (*c*) Anæmia, myxœdema, beri beri, miscellaneous. Cases which would otherwise fall into other groups are included therein with relevant suffix (*a*), (*b*) or (*c*).

Group 3. Congestive cardiac failure at time of electrocardiogram. Group 4 (but not group 5 or 6) is included if in failure.

Group 4. Myocardium showing patchy scarring or fibrosis with at least some atheromatous narrowing of the coronary arteries.

Group 5. Macroscopic myocardial infarction: (*d*) Old infarct, (*e*) Recent subendocardial infarct, (*f*) Recent transmural infarct.

Group 6. Group 5 *plus* any other group factor.

The distribution of the original 102 cases in terms of the clinico-pathological classification is set out in Table I.

For the purposes of quantitative analysis the hearts were also divided into six groups according to ventricular weight, and the incidence of the various pathological groups in each weight is shown in Table II.

TABLE I.

Summary of Clinico-Pathological Distribution: 102 Cases

Class	Total Number	Males	Females	Mean Age (Years)	Remarks
1	24	11	13	60·0	——
2	7	6	1	58·7	3: anæmia 2: electrolytic disturbance 2: anæmia+electrolytic disturbance
3	20	11	9	61·9	14: taking digitalis 10: Group 4 in failure and taking digitalis 1: moderate hypopotassæmia
4	25	18	7	66·5	2: taking digitalis
5	19	14	5	70·0	4: old infarct 6: recent infarct 9: old+recent infarct—1 taking digitalis
6	7	5	2	64·0	All 7 in congestive failure 4: taking digitalis 4: recent infarct 1: old infarct 3: with anæmia 2: electrolytic disturbances
Total ..	102	65	37	—	

ELECTROCARDIOGRAPHY

Recordings were made with a "Both" direct-writing electrocardiogram which fulfilled the criteria of the New York Heart Association (Criteria Committee, 1953) and the American Heart Association (Committee of Electrocardiography, 1954). The frequency response curve was flat to 50–55 cycles per second. Paper speed was 25 millimetres per second with a tested time marker accuracy of ±2%. Standardization used was 1 millivolt=10 millimetres.

A twelve-lead electrocardiogram was recorded using the Goldberger method of augmentation of unipolar limb leads but with a 5000-ohm resistor in each lead to the central terminal (Rappaport and Williams, 1949).

Criteria for nomenclature and measurement of deflections were those defined by the New York Heart Association (1953) and American Heart Association (1954).

Measurements were made with the aid of a "Watson" magnifying light, the equivalent of a conveniently mounted and well illuminated powerful hand lens. Amplitude was measured to the nearest 0·25 millimetres for the *RS-T* segment and to 0·5 millimetres for the *QRS-T* deflection.

No fundamentally original electrocardiographic measurements are proposed, nor perhaps could they be, because no more can be deduced from an electrocardiogram than the method itself will record—a vector and its rate of change. The relation of left ventricular weight to ventricular activation times will be the subject of a separate communication. A review of previous work suggested the following other features as most fruitful for study: (*a*) Maximum precordial *Q* wave (Wilson, *et alii*, 1944. (*b*) *QRS* as measured by maximum *S* and maximum *R* in the precordial leads (Sokolow and Lyon,

TABLE II.

Distribution of Total Ventricular Weight (T.V.W.) in 102 Cases

T.V.W.		Clinico-Pathological Class						Cases (19) Excluded from All Electrocardiographic Study
Group (Grammes)	Number of Cases	1	2	3	4	5	6	
A: >451	14	3	0	5	1	2	3	4: due to bundle branch block (B.B.B.)
B: 351–450	25	1	1	9	7	5	2	3: due to B.B.B. 1: E.C.G. misplaced anterior infarct
C: 301–350	12	0	0	2	5	4	1	2: due to B.B.B. 2: anterior infarct
D: 251–300	22	4	3	2	6	6	1	4: anterior infarct 1: due to B.B.B.
E: 201–250	15	6	2	1	4	2	0	
F: <200	14	10	1	1	2	0	0	Case 60: limb leads only

1949; $SV1+R$ in V5 or V6). (*c*) *RS-T* segment depression and *T* wave amplitude in the precordial lead showing greatest *R* wave.

RESULTS

Electrocardiogram

By definition the "classical" electrocardiographic patterns of bundle branch block and of recent anterior infarction will so distort the ventricular deflection in the precordial leads that such cases must be excluded from the ultimate analysis. Seven cases of bundle branch block and ten of anterior infarction were omitted subsequently on these unequivocal electrocardiographic signs and two more because of inadequate data (Table II). A total of 83 cases was available for full correlation.

QRS Voltage

Precordial $R+S$.—The sum of the deepest *S* and highest *R* wave in the precordial leads is related to total ventricular weight in Figure I.

A definite tendency is seen for the voltage to increase with total ventricular weight, but the scatter is wide. The formula can express no more than a probability of the presence and degree of left ventricular hypertrophy.

Table III analyses the incidence of positive findings according to ventricular weight for values of maximal $R+$ maximal *S* of 35, 40 and 45 millimetres respectively. The result of using Sokolow and Lyon formula ($SV1+RV5$ or V6> 35 millimetres) is included for comparison. The table shows that any value below 45 millimetres (4·5 millivolts) allows too many false positive diagnoses (Total ventricular weight below 250 grammes) for the sign to be reliable. At this level, however, 50% of left ventricles weighing 40% or more above normal were correctly predicted, and only 3·5% (one case) of positive prediction was incorrect. With lesser degrees of hypertrophy the sign was of limited value, as approximately four of every five cases were overlooked, which reduced the over-all figure for correct positive diagnosis to 37%.

The criteria of Sokolow and Lyon provided a slightly lower incidence of correct positive prediction which was of equivocal significance.

In 16 normal cases the maximum sum of precordial $R+S$ exceeded 35 millimetres only once (40·5 millimetres), and in no case did the sum of $SV1+RV5$ or V6 do so. It seems reasonable to conclude that, in the normal adult over 30 years of age, when the maximum $R+S$ (precordial) exceeds 40 millimetres, left ventricular hypertrophy is probably present, and above 45 millimetres it almost certainly exists.

No common factor could be found among the five cases (Table III) in which normal ventricular weight was associated with an $R+S$ between 40 and 45 millimetres. Three were elderly hypertensive females, one a normotensive male

TABLE III.

The Relation of Maximum R+ Maximum S in Precordial Leads to Left Ventricular Hypertrophy Expressed as Total Ventricular Weight (T.V.W.)

T.V.W. Groups (Grammes)	Number of Cases	$R+S>35$ Millimetres		$R+S>40$ Millimetres		$R+S>45$ Millimetres		$SV1+RV5$ or V6>35 Millimetres	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
A: over 451 (80%>normal)	10	8	80	8	80	6	60	4	40
B: 351–450 (40%–80%>normal)	20	13	65	10	50	9	45	7	35
A+B	30	21	—	18	—	15	—	11	—
C: 301–350 (20%–40%>normal)	8	5	62	5	62	2	25	2	25
D: 251–300 (0%–20%>normal)	17	8	47	6	35	3	17	3	17
C+D	25	13	—	11	—	5	—	5	—
E: 201–250	15	7	47	3	20	1	6	1	6
F: 200 or less	13	2	15	2	15	0	—	0	—
E+F	28	9	—	5	—	1	—	1	—

of 63 years with pulmonary tuberculosis and pronounced coronary atheroma, and one an otherwise healthy male of 32 years, who died with peritonitis following appendicectomy. Had assessment been made merely on clinical grounds, the three hypertensive cases would have been classified as "left ventricular hypertrophy", and the incidence of "correct" positive prediction would have been correspondingly improved.

It is evident that, however measured, the amplitude of the *QRS* deviation is increased in certain cases in which left ventricular hypertrophy is not demonstrable, but in which hypertension is a frequent concomitant. The implication then is that either some factor associated with hypertension itself is responsible, or that lesser degrees of left ventricular hypertrophy are not detected by the method of study employed. The latter explanation may be true, although (Figure I) it may be seen that the hypertensive cases tend to show a relatively greater $R+S$ amplitude than other hearts of the same weight. It might be said only that, while left ventricular hypertrophy and hypertension commonly coincide, the terms must not be considered synonymous when applied to electrocardiographic investigations.

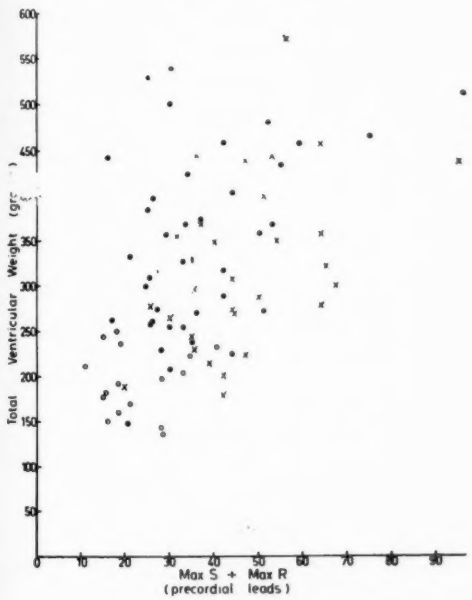

FIGURE I

Maximum $R+S$ (millimetres) in the precordial leads compared with total ventricular weight (83 cases). Circles=normal. Crosses=hypertensive. Dots=others

In fact, as Cabrera and Monroy (1952) suggest, the specific dynamics of the ventricular overload may be of significance.

Finally the influence of the clinico-pathological status on the positive diagnosis (maximum $R+S>45$ millimetres) must be considered (see Table IV).

The figures suggest that advanced ischæmic heart disease with infarction (groups 5 and 6) may reduce the possibility of positive diagnosis, but there is no evidence that congestive cardiac failure, irrespective of digitalis administration (group 3) or lesser degrees of ischæmic disease (group 4), has any similar effect.

TABLE IV.

The Incidence of Maximal $R+S$ (Precordial) Greater than 45 Millimetres in Relation to Clinico-Pathological Status

Clinico-Pathological Group	L.V. Hypertrophy (Weight Group A-D)		Non-Hypertrophy (Weight Group E-F)	
	Total Cases	$R+S>45$ Millimetres	Total Cases	$R+S>45$ Millimetres
1	9	4	15	0
2	4	0	2	0
3	15	7	3	0
4	16	7	7	0
5	9	2	2 } 2	1 } 1
6	2	0	0 }	0 }

Other Voltage Criteria

The value was also studied of various other measurements of R and S wave amplitude which have been proposed in the past.

(*a*) Maximum precordial R wave>26 millimetres (Sokolow and Lyon, 1949). The sign proved positive in 17 of 55 cases of left ventricular hypertrophy, bore a quantitative relation to the degree of hypertrophy, and was negative in all cases of normal ventricular weight. In all 17 cases the maximum sum of $R+S$ (precordial) exceeded 40 millimetres, and in only two was it less than 45 millimetres. The sign may then be of occasional value when $R+S$ lies in the equivocal range of 40 to 45 millimetres.

(*b*) $\frac{R/S \text{ in } V_5}{R/S \text{ in } V_1} > 100$ (Sokolow and Lyon, 1949). The formula has no meaning where R or S is zero. It could be applied to 67 of 83 cases in this series, was positive in 11 of 45 cases of left ventricular hypertrophy and in two of 22 cases of normal ventricular weight. It contributed to diagnosis by the more simple voltage criteria in only one case. The formula is limited in its applicability, is time-consuming to calculate, is liable to gross distortion when the

denominator is less than one and is open to significant false positive prediction. There is no evidence that it offers any useful contribution to the accurate diagnosis of left ventricular hypertrophy.

(*c*) Bipolar limb leads: $R1+S3>25$ millimetres, (Gubner and Ungerleider, 1943). The maximum value of $R1+S3$ in 16 normal cases was 24 millimetres, but it exceeded 25 millimetres in only eight of 55 cases of left ventricular hypertrophy. The sum of maximum $R+S$ in the precordial leads was at least 44 millimetres in all eight cases and exceeded 45 millimetres in six of them; so that the formula made only a very limited contribution to diagnosis.

(*d*) Unipolar limb leads: R in a VL>11 millimetres (Sokolow and Lyon, 1949) or R in a VL>10 millimetres with $T/R<10\%$ (Goulder and Kissane, 1951). Of the 83 cases involved in the present analysis, 56 in which the electrical axis of the heart was horizontal or semi-horizontal (Goulder and Kissane, 1951) were submitted to this test. Patients who had been taking digitalis or quinidine were not omitted from the R-wave analysis, and none was involved in the group (three cases) requiring application of the R/T ratio.

In all, 13 of 38 cases of left ventricular hypertrophy were correctly predicted, which compared with the findings of Sokolow and Lyon (1949) and Scott *et alii*, (1955), but is significantly lower than those of Goulder and Kissane (1951), who claimed correct prediction in 54 of 65 cases. The discrepancy may be attributed to the bias by electrocardiographic selection of cases by Goulder and Kissane. The most significant feature was the high incidence of false positive findings (28%) in ventricles of normal weight, which alone renders the criteria, and particularly the R/T ratio, too unreliable to be of clinical value.

Precordial Q Waves

A prominent precordial Q wave was seen in 51 of the 83 cases under scrutiny. In only two did it exceed one millimetre, and neither in incidence nor in magnitude did it show any real parallel to left ventricular weight. Sokolow and Lyon (1949) also concluded that the precordial Q wave was of no value in the diagnosis of left ventricular hypertrophy.

RS-T Segment Depression

A depression of the S-T segment of less than 0·1 millivolt in the bipolar extremity leads, or 0·075 millivolt in the augmented unipolar precordial leads was classed as normal (Criteria Committee, New York Heart Association, 1953).

In addition to those cases precluded from the voltage analysis, four electrocardiographically "classical" cases of recent posterior infarction were omitted, and independent factors known to depress the S-T segment were considered separately. The latter involved 13 subjects undergoing digitalis therapy and one with proven hypopotassæmia. There remained 65 cases for analysis of S-T segment depression, which was expressed as the percentage found in association with the largest R wave in the precordial "V" leads (Figure II).

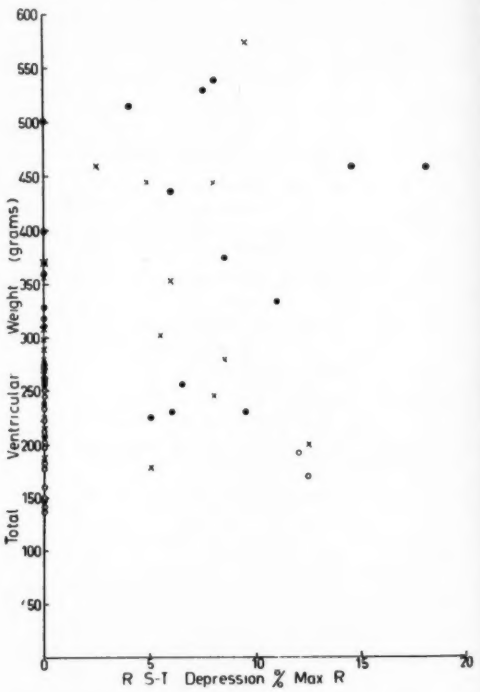

FIGURE II

The relationship between total ventricular weight and S-T-segment depression expressed as percentage of the R wave in the unipolar precordial lead showing the largest R wave. Circles=normal. Crosses=hypertensive. Dots=others

No detailed analysis is required to show that there is practically no correlation between total (left) ventricular weight and S-T segment depression so expressed. No better result was achieved when the maximum S-T depression relative to the R wave in any lead was substituted, nor when the depression was expressed

as an absolute figure. The respective results are analysed in Table V.

It is clear that *S-T* segment depression is common in left ventricular hypertrophy and may even in a very crude fashion increase with its degree, but the significant fact is the number (nine out of 28) of non-hypertrophied ventricles which also show abnormal *S-T* segment depression. Left ventricular hypertrophy then

TABLE V.

The Relationship Between Total Ventricular Weight (T.V.W.) and S-T Segment Depression in the Unipolar Precordial Leads[1]

T.V.W. Group	Number of Cases	Maximal *S-T* Depression Greater than One Millimetre	*S-T* Depression Greater than 5% of Maximal *R* Wave	Maximum *S-T* Depression in Any Lead Greater than 5% of the *R* Wave
A+B	18 (11)	12	9	10
C+D	19 (3)	8	5	8
E+F	28	9	8	9

[1] Digitalis cases excluded are shown in parentheses.

is not primarily responsible for the abnormality, and the relevant factors must obviously be sought elsewhere. In table VI the incidence of abnormal *S-T* depression is related to the clinico-pathological state.

The paucity of cases in the more advanced disease groups (5 and 6) and in the congestive failure group (3) is due to the exclusion of digitalization and recent myocardial infarction.

TABLE VI.

The Incidence of S-T Segment Depression According to Clinico-Pathological Status

Clinico-Pathological Group	Number of Cases	*S-T* Depression Greater than 5% of Maximal *R* Wave
1	24	5
2	5	—
3	5	3
4	22	6
5	8	6
6	1	1

However, the difference between the relatively healthy ventricles (groups 1 and 2) and those with advanced ischæmic or hypertensive disease (3, 5 and 6) is far more striking than was disclosed by weight grouping and is shown by the χ^2 test to be highly significant:

S-T depression > 5% maximum *R* wave
Groups 1 and 2 = 5/29
Groups 5 and 6 = 7/9
$\chi^2 = 5{\cdot}91$, $P < 0{\cdot}02$

When group 3 (congestive failure without digitalization) is included with groups 5 and 6, the significance is enhanced ($P < 0{\cdot}01$).

Lesser degrees of ischæmic disease constitute group 4, in which the incidence of *S-T* abnormality (6/22) did not differ significantly from that in groups 1 and 2.

The distribution of the myocardial disease and of *S-T* segment depression in the total ventricular weight groups is summarized (from Table IV) in Table VII and substantiates the inference that the higher incidence of advanced disease in the larger hearts and not ventricular hypertrophy itself is largely responsible for the frequently associated *S-T* depression.

TABLE VII.

Distribution of Clinico-Pathological Classes in Relation to Total Ventricular Weight (T.V.W.) and Their Influence on the Incidence of S-T Segment Depression in Excess of 5% of Maximum R Wave

T.V.W. Group (Grammes)	Clinico-Pathological Groups			
	1+2	3	4	5+6
A+B (Greater than 350)				
Total number	5	2	6	5
S-T depression greater than 5%	1	1	2	5
C+D (251–350)				
Total number	7	1	9	2
S-T depression greater than 5%	0	1	2	1
E+F (Less than 250)				
Total number	16	3	7	2
S-T depression greater than 5%	4	1	2	1

The Precordial T Wave (V4 to V6)

Changes in the *T* wave may be expressed as a proportion of the associated *R* wave (Sokolow and Lyon, 1949; Goulder and Kissane, 1951; Proger and Minnich, 1935), by absolute standards, or qualitatively, as subnormal, diphasic or inverted. Since the range of *T*-wave normality is wide and the lower limit in V4–6 somewhat tentatively placed at two millimetres, the relative definition may be preferable. Sokolow and Lyon (1949) elected to study the *R/T* ratio and defined the upper limit of normality as 10. The formula cannot be applied when the *T* wave is isoelectric; and when the wave is inverted, additional qualification for the reversal of sign is necessary. The *T/R* ratio is free of these objections, and it was studied in 65 cases. As the converse of Sokolow and Lyon's formula, 10% was taken as the lower limit of normality. Levels selected

for analysis were 10%, 5% and 0 (indicating T-wave negativity) in the precordial lead with the largest R wave. Diphasic T waves were arbitrarily classed as of zero potential.

The scatter diagram (Figure III) shows only very limited correlation between total ventricular weight and the T/R ratio expressed as a percentage.

The impression is that no correlation exists in the case of ventricles within the normal weight range and that it is of crude degree only in the hypertrophied group. Substitution of the minimal T/R ratio in any lead from V4 to V6, irrespective of R-wave voltage, did not improve the over-all pattern.

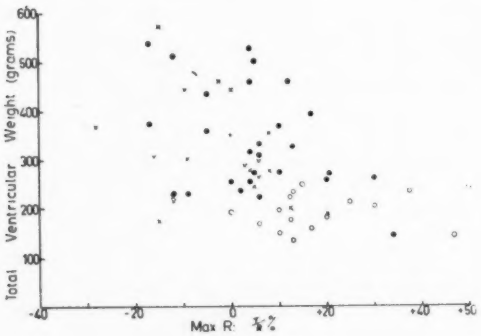

FIGURE III

The relationship between total ventricular weight and the T/R ratio in heads V4 to V6. Circles = normal. Crosses = hypertensive. Dots = others

There is certainly a higher incidence of a subnormal T/R ratio in the hypertrophied than in the non-hypertrophied ventricles, and the difference is significant. The ratio was less than 10% in 30 of 37 cases of hypertrophy and in only 11 of 28 cases of ventricles of normal weight (significance: $P<0.01$). It was less than 5% in 26 hypertrophied and nine "normal" ventricles.

However, when the T/R ratio fell below zero, denoting T-wave negativity, the difference is less apparent—a rather surprising finding if the ratio were directly influenced by the left ventricular mass.

Considerable light is thrown on the apparent anomalies when the T/R ratio is considered in relation to the clinico-pathological state, irrespective of hypertrophy, of the ventricles.

A striking difference was seen in the occurrence of T/R abnormality between the macroscopically healthy (groups 1 and 2) and the severely diseased (groups 5 and 6) or failing (group 3) ventricles and even those ventricles manifesting lesser degrees of ischæmic disease (group 4). The χ^2 test shows the differences to be significant at all three values of T/R:

$T/R<10\%$: Groups 1 and 2 = 12/29
Groups 3, 5 and 6 = 14/14
Significance: $P<0.01$

$T/R<5\%$: Groups 1 and 2 = 10/29
Groups 3, 5 and 6 = 12/24
Significance: $P<0.01$

$T/R<0$: Groups 1 and 2 = 4/29
Groups 3, 5 and 6 = 8/14
Significance: $P<0.01$

The relatively high incidence of advanced disease in the larger ventricles has been discussed earlier (Table II), and it must be concluded that T-wave depression is an indication more of disease than of hypertrophy of the left ventricle. Any further tendency for the T/R ratio to decrease with left ventricular hypertrophy may be explained by the influence of R-wave voltage.

Combined RS-T Segment and T Wave Patterns

There is perhaps no reason to anticipate that the coincidence of RS-T-segment and T-wave abnormality will be more closely related to left ventricular hypertrophy than was each abnormality alone. A brief analysis of the findings confirms such a view.

Of the 65 cases studied, 41 showed a T/R ratio of less than 10% in the precordial lead in which the largest R wave was seen. In 21 of these there was associated depression of the S-T segment of more than 5% of the R wave; in 10 cases actual T-wave inversion was present. In only one case was an S-T depression of 5% found with T/R greater than 10% (12%).

These findings are consistent with the belief of Sokolow and Lyon (1949) that an increase in R/T ratio (decrease in T/R) precedes S-T segment depression; although the ætiology of the changes is clearly not, as they supposed, the direct result of left ventricular hypertrophy.

Of the 21 subjects in which the dual abnormality was found, 11 were in the congestive failure group (3) or in the advanced disease groups (5 and 6), while five suffered lesser degrees of ischæmic disease (class 4). None was taking digitalis.

DISCUSSION AND CONCLUSIONS

The basis of proper evaluation of electrocardiographic patterns attributed to the presence of left ventricular hypertrophy must lie in

analysis of their specificity. Merely to show that left ventricular hypertrophy is often found in the presence of a certain abnormality in the graphic record is not sufficient. The possible occurrence of that abnormality in the absence of left ventricular hypertrophy must also be studied, and the actual frequency with which it occurs when left ventricular hypertrophy exists must be ascertained.

The present study was based on adult patients who reached autopsy at a public general hospital. An electrocardiogram during the last weeks of life was a prerequisite, but this did not imply that all patients suffered from cardiac disease. In fact, in only 36 of 102 cases was death due to cardiac disease, and the presence of recent myocardial infarction in 19 of them necessitated their omission from most of the electrocardiographic study. It is justifiable to claim that conclusions are generally applicable to patients over 30 years of age who are admitted to the medical wards of public hospitals in Australia. The morphological approach does not permit distinction between the dynamic effects of the factor causing left ventricular hypertrophy and the hypertrophy itself. It is possible therefore that conclusions derived from a study of hypertensive individuals should be applied with reservation to, for example, individuals afflicted by rheumatic or congenital valvular disease or hyperdynamic states demanding excessive left ventricular work.

As in most work published in the past, the ætiology of left ventricular hypertrophy in the present investigation was predominantly hypertension.

Of the many electrocardiographic features studied, only those which involved voltage measurements of the *R* and *S* waves were found to be closely related to the presence and degree of left ventricular hypertrophy. The most useful formula was the maximum sum of $R+S>45$ millimetres in the precordial leads, which gave a higher incidence of accurate positive prediction (37%) than did the sum of $SV1+RV5$ or $V6>35$ millimetres (28%) in the same 83 cases. Both formulæ gave one false positive finding amongst 28 ventricles of normal weight.

Even so it appears that the wide range of normality will render it impossible to distinguish lesser degrees of left ventricular hypertrophy even by *R* and *S* voltage criteria. A reduction in the diagnostic level of the sum of maximum $R+S$ introduces false positive findings which cannot be forecast reliably by clinical means. Neither age nor sex nor lesser degrees of ischæmic heart disease and congestive cardiac failure appeared to exert any constant influence on the formula, but advanced ischæmic disease reduced the *QRS* amplitude.

An *R* wave in V5 or V6 exceeding 26 millimetres was found in 31% of 83 cases of left ventricular hypertrophy and in none in which hypertrophy was absent. In general it proved of less diagnostic value than maximum $R+S$ but was sometimes of assistance when this sum lay in the equivocal range of 40 to 45 millimetres. It must be appreciated, nevertheless, that an occasional finding of an *R* wave in V5 or V6 of up to 33 millimetres has been recorded in normal adults (Kossmann, 1953); so that this sign too must be interpreted in the light of probability rather than accepted as inviolable.

The ratio $\frac{R/S \text{ in } V5}{R/S \text{ in } V1}$ is influenced by the electrical axis of the heart as well as by $R+S$ voltage. The incidence of a positive value (>100) was comparable with that found by others (Sokolow and Lyon, 1949; Scott *et alii*, 1955). However, it was positive in a number of cases of normal ventricular weight and cannot be calculated in all cases. Furthermore, it was positive only once when the other voltage criteria were not, so that there seems little purpose in retaining it.

The formula $R1+S3>25$, although apparently specifically associated with left ventricular hypertrophy, was much less sensitive than either of the precordial voltage criteria and has only limited value.

The positive diagnostic value of the *R* wave amplitude, and the T/R ratio, in a VL was found to be outweighed by the incidence of false positive prediction. The necessity must be emphasized for qualification of electrocardiographic findings in the unipolar limb leads, according to the recording technique employed.

RS-T Segment and T Wave

The occurrence of precordial *RS-T*-segment and of *T*-wave depression in left ventricular hypertrophy was analysed in the light of the pathological state of the myocardium, in ventricles of both normal and abnormal weight. As a result it was apparent that the abnormalities were closely related not to the hypertrophy but to the health of the left ventricle.

There are no grounds for the belief that left ventricular hypertrophy is directly responsible for the *RS-T*-segment and *T*-wave abnormalities with which it is often associated. The changes

are a non-specific indication of myocardial disease or disorder which is commonly the result of ischæmia. This statement applies equally to *RS-T*-segment and *T*-wave abnormalities considered alone or together, quantitatively and qualitatively.

The term " left ventricular strain ", if applied to depression of the *RS-T* segment and *T* waves, has little meaning. These changes do not necessarily denote the presence of excessive left ventricular stress, and, if used at all, the term should be applied only when the presence of a high *QRS* voltage denotes left ventricular hypertrophy. Then perhaps the expression (Goldberger, 1949) " left ventricular hypertrophy and strain " may have some prognostic implication. As Roesler and his associates (1940) commented, " it is not entirely clear at the present time what the role is of the hypertrophy, dilatation or myocardial damage in affecting the metabolic activity of the myocardium to produce these final deflection (*RS-T*) changes ". This statement still holds true, and many may agree with Leishman (1951) that ischæmia is the feature that governs the onset of the " strain " pattern in left ventricular hypertrophy. Nevertheless the reversibility of the *RS-T*-segment and *T*-wave depression under many circumstances cannot readily be explained on the simple mechanical basis of the capillary-muscle fibre ratio, even when demonstrable changes in cardiac work are considered(Raab and Lepeschkin, 1950; Miller *et alii*, 1955). While from a clinical view point ischæmic disease will be commonly associated, the biochemical and physiological factors underlying the abnormalities have yet to be defined, and some uncertainty in interpretation will remain until it is achieved.

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BODY FAT, BLOOD PRESSURE AND SERUM CHOLESTEROL OF AUSTRALIAN MEN[1]

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SUMMARY

In a survey of 129 men chosen at random from an industrial organization measurements were made of height, weight, skinfold thickness, circumference of the arm, blood pressure and serum cholesterol concentration. The interrelations between these factors are analysed.

Blood pressure increased principally with age but also with body weight (presumably with obesity, though this was not proven). The serum cholesterol concentration, averaging 244 milligrammes per 100 millilitres, was apparently not related to age, obesity, blood pressure or dietary fat.

Difficulties in assessing obesity and the possible implications of the results of the survey in regard to essential hypertension and vascular degeneration in obesity are discussed.

AUSTRALIA shares with New Zealand, the United States of America, the United Kingdom and several other countries, the great misfortune of having a high and increasing incidence of deaths due to coronary artery disease. The cause of the disease is not known, but of the many factors which have been accused of an ætiological role perhaps the most heavily incriminated, apart from the sexual element which causes males to be affected more often than females, are obesity, hypertension and hypercholesterolæmia. Civilization seems to foster all three of these excesses. On the other hand, it is commonly believed that none of them is at all prevalent among people living in primitive conditions. This is certainly true in New Guinea (Whyte, 1958). But, whereas extensive investigations of blood cholesterol levels have recently been made in many countries in pursuit of the lipid theory of atherogenesis, the other factors have been generally neglected.

Here we have taken advantage of an industrial medical survey being carried out by one of us (I.A.D.), to gather information about obesity, blood pressure and serum cholesterol levels as they exist among Australian men. In addition we were interested to discover whether the malevolent influence of obesity on blood vessels could conceivably be mediated through an effect on either or both of the other two factors, and, indeed, whether these two factors themselves, blood pressure and cholesterol concentration, were in any way related.

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METHODS

Semi-sedentary workers in a transport concern were selected at random according to their union numbers. These men completed a *questionnaire*, which included queries about previous illnesses, present state of health and family history, and attended for interrogation and physical examination.

Height was measured to the nearest half inch, one inch being allowed for shoes. Weight was measured to the nearest pound and included about five pounds of clothing. Subcutaneous fat was measured as the width, to the nearest millimetre, of the double fold of skin and fat which could be pinched up at the mid-point of the posterior surface of the left upper arm. The spring calipers used for this had spherical contacts, and their closing pressure varied with thickness. Blood pressure was measured with an ordinary mercury sphygmomanometer in the left arm with the subject sitting, the diastolic reading being taken at the disappearance of sound. Recordings were made to the nearest even number of millimetres of mercury. The cuff was of standard width and was fitted with metal braces and clip. An index of arm circumference was provided for each subject by noting the particular metal stay which was clipped. Measurements with a tape measure in a series of men allowed of the mean cuff indices being converted to arm circumference. A blood sample was taken and the serum cholesterol

TABLE I.

A Summary of the Principal Findings (Means and Standard Deviations)

Age (Years)	Number	Height (Inches)	Weight (Pounds)	Pounds Weight per Inch of Height (W/H)	Weight for Height[2] (W/H², Kilograms × 1000 ÷ Centimetres²)	Relative Weight (*per Centum*)	Circumference of Arm (Centimetres)	Fatness of Arm (Millimetres)	Blood Pressure (Millimetres of Mercury)		Serum Cholesterol (Milligrammes per 100 Millilitres)
									Systolic	Diastolic	
20–29	11	70·4 (1·81)	164·1 (28·02)	2·324 (0·341)	2·332	106	26·6	7·6 (3·75)	130·0 (12·13)	86·8 (7·08)	246 (39·3)
30–39	52	69·6 (2·62)	168·9 (21·27)	2·423 (0·271)	2·454	106	28·5	9·8 (3·88)	130·3 (10·63)	88·2 (8·75)	235 (43·8)
40–49	38	68·7 (2·56)	165·3 (18·40)	2·409 (0·255)	2·471	105	28·8	10·8 (3·71)	132·5 (12·36)	92·7 (9·54)	248 (46·8)
50–59	27	68·8 (2·86)	171·0 (22·85)	2·484 (0·297)	2·546	107	29·0	10·6 (3·64)	153·6 (23·82)	100·3 (14·08)	254 (46·4)
60–69	1	—	—	—	—	—	—	—	144	84	—
41·7 (9·52)	129	69·2 (2·69)	167·9 (21·74)	2·417 (0·291)	2·461 (0·286)	106	28·4 (2·18)	10·1 (3·88)	136·0 (17·56)	91·6 (11·10)	244 (44·8)

measured by the method of Abell *et alii* (1952). The standard weight for each subject was drawn from the tables of weight for height and age of the Metropolitan Life Insurance Company, due allowance being made for shoes and clothing.

All of the 129 men selected in this random fashion reported for examination. Measurements of subcutaneous fat were commenced later in the series and were made on 88 subjects. Results were analysed by ordinary statistical techniques with the help of an electronic computing machine and accepted as showing significant relationships when the probability of a chance occurrence was less than 5%. The suffixes †, ‡, § have been used here to indicate probabilities of less than 5%, 1% and 0·1% respectively.

RESULTS

A summary of the findings in the different age groups is given in Table I and the overall correlation matrix in Table II.

Body Build

Height.—The average height was 69·2 inches. Younger men were distinctly taller than older men; the correlation coefficient of height with age was −0·207*. This is commonly found in surveys such as this and is attributed to the fact that the height of succeeding generations is increasing as well as to the shrinkage which occurs with advancing age.

Weight.—The average weight, including five pounds of clothing, was very close to 12 stone (167·9 pounds). There was no apparent

TABLE II.

Correlation Coefficients for the 129 Subjects (88 with Measurements of Fatness of Arm).[1]

——	Age	Cholesterol	Blood Pressure			Arm Circumference	Height	Weight	W/H	Fatness of Arm
			Systolic	Diastolic	Mean					
Cholesterol	+0·188*	—	—	—	—	—	—	—	—	—
Systolic blood pressure	+0·438§	+0·124*	—	—	—	—	—	—	—	—
Diastolic blood pressure	+0·383§	+0·207†	+0·698§	—	—	—	—	—	—	—
Mean blood pressure	+0·450§	+0·170*	+0·954§	+0·881§	—	—	—	—	—	—
Arm circumference ..	+0·208†	+0·100*	+0·184*	+0·290‡	+0·244†	—	—	—	—	—
Height	−0·207†	+0·043*	−0·034*	+0·031*	−0·011*	−0·041*	—	—	—	—
Weight	+0·008*	+0·064*	+0·286‡	+0·334§	+0·330§	+0·514§	+0·446§	—	—	—
W/H	+0·051*	+0·039*	+0·301‡	+0·333§	+0·340§	+0·575§	+0·150*	+0·927§	—	—
Fatness of arm ..	+0·124*	+0·163*	+0·224†	+0·057*	+0·170*	+0·429§	+0·300‡	+0·560§	+0·492§	—
W/H²	+0·300‡	−0·016*	+0·360§	+0·288‡	+0·352‡	+0·591§	−0·527§	+0·488§	+0·917§	+0·437§

* not significant. † 5%. ‡ 1%. § 0·1%

relationship to age. Weight increased on the average about three and a half pounds for every inch increase in height.

Weight in Relation to Height.—Weight alone is not very informative about the bulk of the body, being dependent on height as well as on other dimensions of the body. Weight per inch of height, W/H, sometimes called the Ponderal Index, is a better comparative measure of bodily bulk, but is usually found to vary with height and therefore to be unsatisfactory for comparing groups which differ markedly in height. In the present series this Ponderal Index showed no significant relation to height or age but was strongly correlated, as would be expected, with the circumference of the arm (r=0·575‡).

The index of weight in relation to the square of the height, W/H^2, is claimed to be a better comparative measure of bodily bulk because it is said to be independent of height (Boe *et alii*, 1957). In the present series, however, this has not been the case. Squaring the height has over-compensated for the influence of height on weight, as is shown by the highly significant negative correlation (−0·498‡) between W/H^2 and height even when the effect of age has been excluded. Bodily bulk, as defined by this index, increased with age (r=+0·230*, allowing for changes in height with age) and was clearly reflected by the circumference of the arm (r=0·591‡).

Perhaps the commonest method for expressing bodily bulk is in relation to tables of standard weight for height and age, such as those of the Metropolitan Life Insurance Company. Our Australian men were slightly overweight by this scale, 106% of standard, at all ages. Since the tables allow for increasing weight with age, the results mean that these Australian men increased in weight at the same rate as the American subjects from whom the tables were compiled.

Fat on the Arm.—The average measurement of the double fold of skin and fat which could be pinched up from the back of the arm was 10·13 millimetres. Its apparent rise with age was not significant, but it was strongly related, as might be expected, to all estimates of bodily bulk—weight (r=0·560‡), W/H (0·492‡), W/H^2 (0·437‡) and arm circumference (0·429‡). The correlation with W/H^2 became even stronger (0·721‡) when due allowance was made for variations in height and age within this group of subjects. A surprising finding was that the thickness of fat apparently increased with the height of the individual; the partial correlation coefficient was 0·262* when correction was made for variation in W/H, and even higher, 0·694‡, when W/H^2 was held constant.

Blood Pressure

The data were examined for the relation between blood pressure and age, indices of body build and serum cholesterol.

Age.—Both systolic and diastolic pressures rose with age. This association was strongly positive even when allowance was made for variations in height, weight and arm circumference (r=0·443‡ for systolic and 0·442‡ for diastolic pressure).

Body Build.—There was no apparent relationship between blood pressure and height nor was there any suggestion that arm circumference was influencing the blood pressure reading (r=−0·098 for systolic and +0·048 for diastolic pressure, age and W/H being held constant).

Evidence in support of an association between pressure and bodily bulk was conflicting. Thus, with the influence of variations in age, height and circumference of the arm eliminated, the total body weight showed a strong correlation with systolic pressure (r=0·315†) and with diastolic pressure (0·269†). A similar association existed between the ponderal index, W/H, and blood pressure: r=0·307† for systolic and 0·272† for diastolic pressure, with age and circumference of the arm held constant. The simple correlation coefficients for W/H^2 and blood pressure seemed to confirm this, but their significance was considerably reduced, if not entirely eliminated, when allowance was made for the effect of age and circumference of arm (r=0·252* for systolic and 0·081 for diastolic pressure). Further confusion comes from examining the relation between blood pressure and the measure of fat on the arm. The straightforward correlation coefficients suggested a positive association between fat and systolic pressure (0·224*) but not diastolic pressure (0·057). However, the partial correlation coefficients which emerged after making allowances for the independent influences of age, height and weight on blood pressure suggested that fatness and systolic pressure were unrelated (0·026), while diastolic pressure tended to fall as fatness increased (−0·228*)!

Table III shows the relative importance of age, height and the various measures of bodily bulk as well as circumference of the arm in so far as they affect blood pressure. Thus, the best fitting linear relation for systolic blood pressure expressed in terms of height, weight and age is as follows:

(Systolic pressure) = −0·0926 (height) +0·3240 (weight) +0·4157 (age) where each of the variables is expressed in its "normalized"

TABLE III.

Regression coefficients for the best-fitting linear relationships between normalized variables when blood pressure is expressed in terms of (a) *height, weight and age,* (b) *weight per unit of height and age,* (c) *weight per unit of height, age, skinfold and arm-circumference,* (d) *weight per unit of height², age, skinfold and arm-circumference. The normalized form of a variable V is* $\frac{V-Vm}{Vs}$, *where Vm is the mean and Vs its standard deviation. The correlation coefficient, r, is a measure of the precision with which the straight line fits the observations*

Blood Pressure	Height (H)	Weight (W)	W/H	W/H²	Age	Skinfold	Circumference of Arm	r
(a) Systolic	−0·0926	+0·3240	—	—	+0·4157	—	—	0·278
Diastolic	−0·0496	+0·3529	—	—	+0·3697	—	—	0·258
(b) Systolic	—	—	+0·2798	—	+0·4234	—	—	0·230
Diastolic	—	—	+0·3147	—	+0·3668	—	—	0·245
(c) Systolic	—	—	+0·3131	—	+0·4375	+0·0644	−0·1143	0·279
Diastolic	—	—	+0·3632	—	+0·3768	−0·2086	+0·0932	0·280
(d) Systolic	—	—	—	+0·2568	+0·3665	+0·1048	+0·0890	0·260
Diastolic	—	—	—	+0·1278	+0·3181	+0·1248	+0·2018	0·210

form, that is as deviation from its mean divided by the standard deviation. Using this normalized scale allows of the regression coefficients, which are closely analogous to partial correlation coefficients, being used directly as measures of the relative importance of each item. Table III emphasizes that age and weight (or weight in relation to height) were the major determinants of blood pressure and that age exerted a relatively greater influence than weight.

Table III also shows that the prediction of blood pressure from the height, weight and age of the subjects in this series was practically as good as when any of the other indices were used, including fatness and circumference of the arm. Expressed in ordinary units the equations become :

Systolic pressure (mm. Hg)=101·886 −0·604 height (in.)+0·262 weight (lb.)+0·767 age (yr.)

Diastolic pressure (mm. Hg)=57·453 −0·204 height (in.)+0·180 weight (lb.)+0·431 age (yr.)

Serum Cholesterol.—There was no evidence that individuals with raised levels of serum cholesterol also had raised blood pressure. Any possible influence of age and fatness having been eliminated, the correlation coefficients for serum cholesterol level and blood pressure were 0·149 for the systolic and 0·021 for the diastolic pressure.

Serum Cholesterol

The overall average serum cholesterol content was 244 milligrammes per 100 millilitres with a standard deviation of 45 milligrammes per 100 millilitres. There was no definite trend with age ; the simple correlation coefficient of 0·188 was little changed by eliminating any influence of obesity (W/H and fatness of arm). Nor was there any evidence of the serum cholesterol level being related to the bulkiness of the body. The correlation coefficient with W/H was −0·048, and with W/H² −0·153, age and girth of arm being kept constant. Nor was there any demonstrable relationship with fatness, the skinfold thickness being taken as a measure of the total body fatness : r=0·148, with age and W/H constant. Nor, as mentioned previously,

TABLE IV.

A Comparison of Diet and Other Measurements in Two Groups, Each of 10 *Subjects, with Serum Cholesterol Concentration below* 200 *Milligrammes per* 100 *Millilitres in One and above* 300 *Milligrammes per* 100 *Millilitres in the Other*

Group	Serum Cholesterol (Milligrammes per 100 Millilitres)	Age (Years)	Height (Inches)	Weight (Pounds)	Arm Fat (Millimetres)	Relative Weight (Percentage of Standard)	Blood Pressure (Millimetres of Mercury)		Daily Diet			
							Systolic	Diastolic	Total Calories	Fat (Grammes)	Fat (Percentage of Calories)	Cholesterol (Milligrammes)
A	158–192	39·8	70·8	181·8	9·0	109·6	134	90	3274	144	39	894
B	300–349	47·5	68·8	166·7	11·4	104·8	136	93	3022	130	38	944

was there any significant correlation between serum cholesterol level and blood pressure.

An assessment of the diet was made in 10 men with serum cholesterol levels of 300 milligrammes per 100 millilitres or higher and in 10 with levels below 200 milligrammes per 100 millilitres. The results are shown in Table IV. The groups with lower levels of cholesterol were slightly younger, taller and heavier, but their diet was substantially the same as for the other group.

DISCUSSION

In the characteristics measured in this study Australian men appear to be comparable with other Europeans. Their relative weight at all ages is similar, their blood pressure is of the same order and increases with age, and the average level of serum cholesterol is high. Little wonder then, if there be any justification for the suspicions entertained of obesity, hypertension and cholesterol, that their risk of succumbing to cardio-vascular diseases is also high. It is easy to conceive of hypertension causing cardio-vascular trouble, and plausible hypotheses have been put forward to implicate blood cholesterol. But, if we assume that obese people are truly more prone to develop cardio-vascular diseases, it is not clear why obesity should lead to pathological changes in blood vessels. Genetic and metabolic factors may be involved, or the effect of obesity may be mediated through changes in blood pressure or serum cholesterol. However, attempts to unravel these mysteries always encounter the difficulty of measuring obesity (Keys and Brozek, 1957) and the confusing effect of a fat arm on the measurement of blood pressure (Pickering, 1955).

Obesity

The only true measure of obesity would come from measurements of total body fatness, but the methods are not practicable when one is dealing with large numbers of subjects. All other methods of assessment are inferior and subject to error and misinterpretation, as is emphasized by Keys and Brozek (1957). Estimates based solely on body weight or weight in relation to height will readily detect extreme degrees of obesity or emaciation, but will be unreliable measures of smaller variations in obesity because of the variable proportions of fat and muscle in different individuals. Moreover, differences in height and shape of individuals introduce difficulties which cannot be completely eliminated by referring to standard tables or by using such ratios as weight over height or weight over height^2. Perhaps one of the most commonly used measures of obesity, and one of the most misleading is the Relative Body Weight derived by expressing the actual weight as a percentage of a "standard", "desirable" or "ideal" weight.

Tables of standard weights, however, are really average weights of apparently normal individuals of different heights and ages. They accept as normal, or at least as "standard" or "desirable", an increasing weight with age, which is indicative of increasing obesity. This point is illustrated in Figure I, where the data for Australian men are compared with observations made among natives in New Guinea (Whyte, 1958). As judged by the relative weight scale, Australian men were slightly

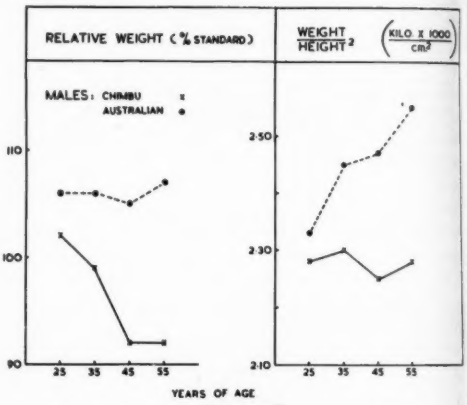

FIGURE I

A comparison of the bodily bulk of males in Australia and New Guinea using two different indices

overweight, but to the same extent at all ages; whereas the natives became progressively more underweight with advancing age. It might be mistakenly assumed from this that the degree of obesity of Australians remained constant, while that of the natives decreased with age. In fact, the reverse is probably true, as can be seen by taking weight over height^2 as the index of bodily bulk. On this scale the natives remained constant, while the Australians increased markedly in bulk, and presumably in obesity, with age. The special virtue of this index is that it is said to provide a reliable comparative index of bulk which is independent of height (Bøe *et alii*, 1957); in our own experience this was true for male subjects in New Guinea but not for the Australians in the present study.

If we assume that the body is cylindrical and of uniform consistency, then constancy of W/H^2 with varying H would imply that the width of the body varies directly with height. Constancy

of W/H on the other hand would imply that bodies of different heights have the same width. No simplified index of bulk is likely to be universally applicable. Our present data would suggest something like $W/H^{1.25}$ for the men in the present series.

Even if it is not possible to measure the total fat, it is obviously desirable to measure some part of the bodily fat, and it is in this regard that skinfold thicknesses have been used. Precision depends on the number and location of sites chosen and assumes that subcutaneous fat bears some definite relation to the total fat content of the body. Earlier work suggested that the triceps was a satisfactory site, though less satisfactory than taking the sum of measurements at several sites (Keys and Brozek, 1953), but further observations have shown that its validity decreases with age (Brozek, 1957). Our own observations in New Guinea tend to confirm this. Therefore, the present study would have been more informative had we made measurements at several sites; and some of the discordant results obtained from our skinfold measurements, as compared with other estimates of obesity, may be due to the site we used.

The net result is that none of our estimates of obesity in the present study is entirely reliable; but weight, weight over height, weight over height^2, arm fat and the circumference of the arm are all positively related one to another.

Blood Pressure

Blood pressure indubitably increases with age. This confirms what has generally been found among Europeans and other races. Nevertheless, a rising pressure is not an inevitable feature of aging, as was shown by observations in New Guinea (Whyte, 1958), and it is presumptuous to accept a rising pressure with age as normal. It is probable that the "ideal" pressure, like "ideal" weight, is to be found among healthy young adults, and that any rise with age is hazardous and should be regarded as pathological. If we take this view, essential hypertension is a disease, albeit one which afflicts the majority of aging civilized beings, and its cardinal sign is a blood pressure substantically exceeding the normal 120/80 mm. Hg. This is a reversion to the older conception of the disease and a denial of the recent tendency to raise, in step with age, the level of blood pressure which arbitrarily divides normotension from hypertension (Master *et alii*, 1952). Whereas Pickering (1955) and his colleagues regard essential hypertension as the "upper end of frequency distribution curves showing continuous variation", the concept put forward here is that the essence of the disease is the gradual shift with age of the whole distribution curve towards higher pressures. Even many of the individuals in the lower end of the curve are afflicted (though less likely to suffer) in so far as they are participating in this mass movement associated with aging.

Our thinking along these lines has been conditioned to some extent by our interpretation of findings among natives in New Guinea, which is rather insecure evidence in so far as it pertains to only one race of people whose diet and pattern of diseases we might regard as substandard. But the concept gains strength from the fact that it is really an extension of the Pickering concept. We agree that there is a quantitative and not qualitative difference between individuals with lower and those with higher blood pressures; that inheritance plays a part in determining the place of an individual within the distribution curve; and that sex and environmental factors must be important. But instead of accepting as normal the upward movement of pressure with age and concentrating attention on the factors operating within the distribution curve, we would regard the age effect with suspicion and extend our investigations to embrace it. The concept invites a wider focus for research; it certainly should not be seen as a signal for imposing the punishment of present-day treatment on the hordes of "benign hypertensives" so defined.

Blood pressure also increases with bodily bulk. This conclusion has been reached in most of the published surveys, but there has always been some hesitation in fully accepting it because of the possibility that the raised pressures observed in the obese were due to the errors introduced when one is dealing with thick arms (Pickering, 1955). Moreover, increasing bulk is often associated with increasing age, and the effects of these two factors are not always clearly separated. Bøe *et alii* (1957) went so far as to conclude from their very extensive survey in Norway that the pure effect of weight on blood pressure was very small when the completely dominating influence of age was eliminated, and perhaps negligible if the error due to arm circumference could be subtracted. Our present study has taken cognizance of each of these variables and reveals that age is, indeed, the major factor affecting blood pressure, but weight is, nevertheless, a very real one. The relative importance of age and weight can be seen from the changes which would lead to a rise of 10 mm. Hg in the systolic and diastolic pressures as predicted from the regression on height, weight and age (Table V).

These are much the same as predictions based on our other regression equations, even if corrections are made according to the method of Pickering, Roberts and Sowry (1954) for the possible effect of arm circumference on blood pressure readings. They suggest that the blood pressure of Australian men rises less steeply with age but more acutely with increased body weight than is the case with Norwegians.

Another way of illustrating our results is to predict the changes in blood pressure which would accompany an increase in body weight, if we assume that the findings of the survey apply to a single individual gaining weight. If a man 30 years of age and 5 feet 10 inches in height suddenly increases in weight from 10 stone to 15 stone, his blood pressure would be

TABLE V.

Increase of 10 Millimetres of Mercury	Increase in Weight (Pounds)	Increase in Years (Age)
Systolic pressure	38	13
Diastolic pressure	55	23

expected to rise from 119/84 to 138/96 mm. Hg —that is, from 122/76 to 133/84 mm. Hg if the Pickering correction is made for the estimated circumference of the man's arm. But whether the effect on blood pressure is attributable solely to obesity or simply to total weight, be it made up of muscle or fat, is undecided and must await further study. In the present study no clear relationship was demonstrated between blood pressure and " fatness ", the thickness of fat on the arm being taken as a measure of total body fatness, beyond a doubtful negative relation with the diastolic pressure. However, the significance of this is impaired by the inadequacy of the arm skinfold as an index of fatness, as was discussed earlier.

This tendency of blood pressure to increase with bodily bulk might also be regarded as a feature of essential hypertension. Certainly age and weight influence the blood pressure of Australians and other Europeans. By contrast no association between blood pressure and body weight was detected among the natives of New Guinea.

Serum Cholesterol

The serum cholesterol level of the Australian men in this series, 244 milligrammes per 100 millilitres, is of the same order as that reported from the United Kingdom and America. In many surveys the level has been found to increase with age, but absence of this trend in the present study is in agreement with what Adlersberg *et alii* (1956) found for men between the ages of 33 and 60 years in New York. There is general agreement that an individual's serum cholesterol level increases while he is putting on weight, but conflicting opinions have been expressed regarding the relationship of serum cholesterol level to a steady state of obesity. Tanner (1951) is among the few workers who have found a positive correlation. He found a significant relationship between cholesterol level and body weight and between the serum level and obesity as judged by skinfold thickness. The skinfold over the triceps, as used in our own study, was much inferior to the subcapsular site. The majority of observers, however, have reported no significant correlation (for example, Hobson *et alii*, 1953; Keys and Keys, 1954; Lindholm, 1956). Similarly, the lack of association between cholesterol level and blood pressure in the present survey is in agreement with other reports (Malmros *et alii*, 1956; Hobson *et alii*, 1953).

In fact the levels of serum cholesterol in these men were not related to any of the other characteristics which were noted—neither to age, obesity or blood pressure, nor to diet. The limited inquiry into their dietary habits suggested that the possession of a high or low level of serum cholesterol could not be attributed to differences in fat consumption. The observed range of serum values is probably the normal scatter for males eating a moderately high-fat diet, with heredity playing an important part in determining the place of each individual within the scatter.

If one had to choose between blood pressure and serum cholesterol level as playing a part in the encouragement of atheroma in obese people, the evidence would seem to favour blood pressure.

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SERUM CHOLESTEROL LEVELS OF AUSTRALIANS AND NATIVES OF NEW GUINEA FROM BIRTH TO ADULTHOOD[1]

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SUMMARY

The serum cholesterol concentration of newborn infants was the same for Australians (70 milligrammes per 100 millilitres) as for natives (68 milligrammes per 100 millilitres). Measurements were also made of phospholipids, total fatty acids and lipoprotein fractions for Australian newborn infants and their mothers. There was no significant correlation between the lipid levels of mother and child.

The cholesterol concentration doubled within one year in both races and then rose steadily with age in Australians and fell gradually in the natives to adult levels. Possible causes of this acquired deviation are discussed, especially the differences in dietary fat and protein.

ONE of the few attributes of the primitive natives of New Guinea which are envied by civilized people is their relative freedom from coronary heart disease. The reason for their immunity is not at all clear, though contributory factors may include their general lack of obesity and hypertension (Whyte, 1958) and their low level of serum cholesterol (De Wolfe and Whyte, 1958). The latter is no doubt largely due to their diet, which contains very little protein and almost no fat, but genetic constitution, liver disease and other acquired ailments may play a part. It would clearly be of interest to learn whether the difference in serum cholesterol levels of the natives and Europeans is already established at birth, or whether it is acquired during growth to adulthood. This we have been able to do in the investigation reported here.

There has been general agreement among previous studies in European subjects, as reviewed by Rafstedt (1955), that serum lipid concentrations are low in the newborn infant, rise rapidly towards adult levels within a few days and then show little or no change with age during childhood and adolescence. The average serum cholesterol concentration at birth has been found to be 70 to 80 milligrammes per 100 millilitres. Newborn African and European infants were found by Bersohn and Wayburne (1956) to have the same cholesterol levels.

METHODS

Cholesterol was measured by the method of Abell *et alii* (1952). Most of the samples of serum had been stored frozen, but fresh samples were obtained from the umbilical cords of 25 Australian babies and from some of their mothers. Other measurements which were made in some of the samples of fresh serum included estimations of phospholipids by the method of Zilversmit and Davis (1950), of total fatty acids by the method of Stern and Shapiro (1953) and of lipoproteins[1] by paper electro-

TABLE I.

Lipid Values in the Serum of Newborn Australian Infants and Some of their Mothers

Substance	Newborn Infants		Mothers	
	Number of Subjects	Mean and Standard Deviation	Number of Subjects	Mean and Standard Deviation
Fatty acids (milligrammes per 100 millilitres of serum)	6	59·5 (15·2)	9	142·6 (70·1)
Cholesterol (milligrammes per 100 millilitres of serum)	25	70·0 (19·3)	19	276·6 (64·6)
Phospholipid (milligrammes per 100 millilitres of serum)	12	114·6 (21·2)	12	324·3 (58·9)
Cholesterol/phospholipid ratio	12	0·61 (0·134)	12	0·81 (0·104)
Alpha lipoprotein (milligrammes of cholesterol per 100 millilitres of serum)	6	31·0 (5·2)	6	61·8 (25·6)
Beta lipoprotein (milligrammes of cholesterol per 100 millilitres of serum)	6	49·8 (25·3)	6	207·7 (51·7)
Beta lipoprotein (percentage of total cholesterol)	6	59·1 (10·48)	6	77·0 (7·09)

[1] Received on April 15, 1958.

[2] Director.

[3] Technical Assistant.

[1] We are grateful to Dr. R. B. Goldrick for these estimations.

phoresis and elution of cholesterol (Langan *et alii*, 1955).

The serum from natives in New Guinea was collected in the Chimbu area of the Central Highlands by Dr. P. S. Venkatachalam[2] and generously made available to us by him and by the Red Cross Blood Transfusion Service in Sydney. The Red Cross also supplied samples of serum from Australian children and young adults. Serum from Australian newborn infants and their mothers was kindly collected by Dr. A. C. Christie at the Royal Hospital for Women, Sydney. The numbers of subjects involved are shown in Tables I and II.

TABLE II.

Serum Cholesterol Levels (mg. per 100 ml.) at Different Ages in Australians and in Natives

Age (Years)	Australia		New Guinea	
	Number	Mean and Standard Deviation	Number	Mean and Standard Deviation
Newborn	25	70 (19·3)	13	68 (17·0)
0 to 1	6	145 (39·9)	6	151 (36·6)
2 to 5	12	167 (48·8)	13	142 (38·7)
6 to 10	13	164 (34·8)	24	141 (29·1)
11 to 15	15	186 (50·4)	21	134 (38·1)
16 to 20	18	185 (33·1)	20	122 (39·7)
20 to 29	46	205 (45·3)	53	130 (33·2)

As no differences in cholesterol levels of males and females have been found in this small series, which is in accord with experience elsewhere (Rafstedt, 1955), measurements in both sexes have been pooled.

RESULTS

The cholesterol concentration in blood from the umbilical cord of newborn babies is the same for Australians (70 milligrammes per 100 millilitres) as for natives in New Guinea (68 milligrammes per 100 millilitres).

The concentrations of cholesterol and other lipids in fresh serum from Australian newborn babies and their mothers are shown in Table I. The lipid values are lower in the newborn. The difference is most marked in respect to cholesterol (70 as opposed to 277 milligrammes per 100 millilitres), being due almost entirely to cholesterol carried in the beta lipoprotein fraction. Cholesterol and phospholipid levels are closely correlated in the newborn ($r=+0{\cdot}699$; $n=12$; $0{\cdot}2>p>0{\cdot}1$) and even more so in the mothers ($r=+0{\cdot}829$; $n=12$; $p<0{\cdot}001$). There is no apparent association between the maternal and infant serum levels of cholesterol ($r=+0{\cdot}199$; $n=19$; $p>0{\cdot}1$) or of phospholipid ($r=+0{\cdot}013$; $n=12$; $p>0{\cdot}1$).

The cholesterol concentrations found at different ages up to adulthood are shown in Table II and Figure I. In both Europeans and natives there is a sharp and equal rise within the first year of life. Thereafter, the level in Australian children appears to climb steadily with age towards the value found in young adults (205 milligrammes per 100 millilitres), while the level in the natives slowly falls to the average adult concentration of 130 milligrammes per 100 millilitres.

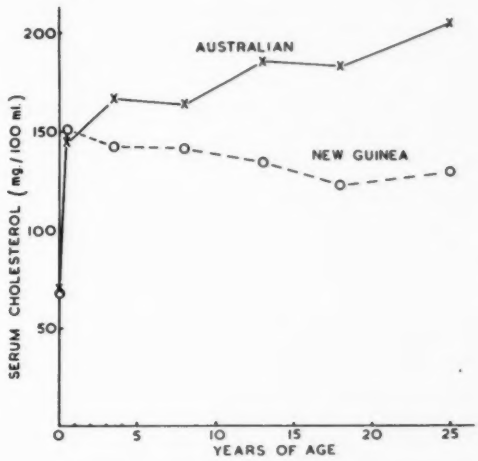

FIGURE I

Changes in serum cholesterol concentration with age in Australians and natives of New Guinea

DISCUSSION

The low levels of lipids in the serum of Australian newborn infants are similar to those found in other studies among Europeans. Rafstedt (1955) has given a comprehensive review of the literature on the subject and himself found a total cholesterol of 74 milligrammes per 100 millilitres, a phospholipid concentration of 124 milligrammes per 100 millilitres, and a low level of beta lipoprotein. Russ, *et alii* (1954) reported similar results (cholesterol 68 milligrammes per 100 millilitres, phospholipid 124 milligrammes per 100 millilitres) and found by Cohn's fractionation that 55% of the cholesterol was present in the beta

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lipoproteins (fractions (i) and (iii)). The ratio of cholesterol to phospholipid in the beta fraction was lower (0·87), than in pregnant (1·07) or non-pregnant (1·25) women, and the authors suggested that the pattern of lipids in the infant might in part be due to the influence of œstrogens. Our own results were very similar to these—cholesterol 70 milligrammes per 100 millilitres, phospholipid 115 milligrammes per 100 millilitres, and 59% of the cholesterol in the beta fraction. Adult male Australians normally carry 78% of the cholesterol in the beta fraction (de Wolfe and Whyte, 1958).

Our figures for lipids in the serum of mothers immediately after delivery of their babies are rather higher than the average levels for women and probably reflect the changes which are known to occur during pregnancy (Oliver and Boyd, 1955). Senn and McNamara (1937) have suggested that the lipids of the newborn are derived from the maternal circulation, but it seems unlikely that the placenta would allow them to pass (Popjak, 1954). Moreover, the concentrations in the newborn infants of the present series showed no dependence on the maternal levels, and a similar lack of correlation has been computed from the measurements made by Russ *et alii* (1954).

The newborn infants of Australia and New Guinea have the same concentration of cholesterol in serum, and, if we judge by the uniformity of results obtained in studies of other populations, there is no reason for believing that they differ in respect to other lipids such as phospholipids and the distribution of lipoproteins. Bersohn and Wayburne (1956) found a total cholesterol level in African newborn infants and their mothers of 71 and 190 milligrammes per 100 millilitres respectively, while the corresponding figures for Europeans were 75 and 258 milligrammes per 100 millilitres. Europeans derive 35% to 40% of their calories from fat, Africans about 20% and the natives of New Guinea less than 5%. Lipid levels in the newborn appear to be independent of race and of the diet and serum levels of the mother.

The increase in serum cholesterol level during infancy and early childhood is the same for Australians as for natives in New Guinea and probably follows the pattern which has been traced in greater detail elsewhere. For example, Sperry (1936) found serum cholesterol levels of 50 to 70 milligrammes per 100 millilitres at birth and 70 to 190 milligrammes after four to 25 days; Senn and McNamara (1937) found a level of 80 milligrammes at birth and 135 milligrammes in infants six to ten days old; and Rafstedt (1955) observed a rise from 74 milligrammes at birth to 134 milligrammes per 100 millilitres of serum in three to ten days. Most of the increase appears to take place within the first four days of extra-uterine existence and is at least partly due to the nature of the food consumed. Rafstedt found an increase of only 3 milligrammes (per 100 millilitres of serum) when infants were fed solely on carbohydrate for their first three days of life, whereas the increase was 31 milligrammes when a mixture of carbohydrate and amino acids or fat-poor citrated milk was used, and 60 milligrammes when normal milk was given. Pomeranze (1956) found that the serum cholesterol level rose less in infants fed on soy milk than in those fed on cow's milk, which he attributed to the difference in saturation of the fatty acids.

Venkatachalam (1957) has made a detailed study of the nutrition and growth of children in the Chimbu area of New Guinea. Native children are exclusively breast-fed for six months, and half of them are still deriving some of their nourishment from breast milk at four years of age. The milk contains the same concentration of protein but only half the amount of fat found in European maternal milk. The children show a slower increase in weight and in height than Australians, but this may be a racial characteristic apart from any influence of diet. At no stage is there any significant amount of fat in their diet, apart from what is provided in breast milk, and the intake of protein increases gradually to the adult consumption, which is only about 30 grammes daily. Infestation with worms is common, as are respiratory diseases, gastro-enteritis, malnutrition and liver disease. However, hæmoglobin levels are the same as in New South Wales, and the total protein concentration is normal, though serum albumin tends to be low.

In later childhood the Australian cholesterol levels continue to rise, perhaps in response to a high intake of protein and fat, while the levels in the natives fall slowly to the adult level of 130 milligrammes per 100 millilitres of serum. In general, other studies have revealed no particular trend with age (Hodges *et alii*, 1943; Rafstedt, 1955; Adlersberg *et alii*, 1956) but Scrimshaw *et alii* (1957) have shown that the serum cholesterol level in children stands in relation to social class and the dietary intake of fat in the same way as it does among adults. Thus, it would seem that Australians and natives of New Guinea, and probably people of all races, begin their lives with the same lipid pattern and then gradually acquire any differences which are apparent among the adult populations. The

reasons for the divergence are obscure. Diseases and infestations may play a part. So may liver disease, though Walker and Arvidsson (1954) found no significant difference in the concentration of cholesterol in the serum of Africans with and those without biochemical evidence of hepatic dysfunction. Bersohn and Wayburne (1956) suggested that the low cholesterol level of natives might be due to hormonal imbalance caused by faulty hepatic function, even without the usual biochemical evidence, which in turn is due to a deficiency of protein. However one looks at the problem, the racial differences in serum lipids can, on the basis of available evidence, more reasonably be attributed to diet than to any other environmental factors. Rigid genetic differences seem unlikely, since the published experience among Indians, South Africans and other races with naturally low cholesterol levels has been that the levels approach European standards in communities eating a European type of diet. Fat and perhaps protein would seem to be the important dietary elements.

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THE PARTIAL ABSORPTION TEST: A NEW TECHNIQUE FOR TITRATING RH ANTIBODIES OF PATHOLOGICAL SIGNIFICANCE[1]

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SUMMARY

A new test is described which enables the quantity of Rh antibody free in the fœtal serum to be estimated by titrations carried out on the maternal serum. Equal volumes of a hæmolysed 25% suspension of Rh-positive red cells and a 1 : 4 dilution of the maternal anti-Rh sera are incubated at room temperature for 15 minutes. The Rh antibody remaining free at the end of this time is titrated by the indirect anti-globulin method. The titre value obtained is referred to as the partial absorption (P.A.) titre.

The P.A. titre is closely related to the titre of free Rh antibody in the cord serum of the infant at birth. The P.A. titre also is highly correlated with the blocking titre, but shows no relationship with the conglutination or albumin titres. The P.A. test, however, is more sensitive than the blocking test in detecting Rh antibodies of pathological significance, and is likely to be therefore of greater prognostic value.

THE main aim of serological tests carried out on the Rh-immunized mother during pregnancy is to provide the obstetrician with a reliable indication of the severity of the hæmolytic process in the fœtus. Ideally he would like to know before the infant is born whether it will be mildly or severely affected; whether, if pregnancy is allowed to continue to its natural term, the fœtus will be liveborn or stillborn; and whether, if the father has been shown to be a heterozygote, the fœtus is Rh-positive or Rh-negative.

Previous work from this laboratory (Kelsall and Vos, 1952; Kelsall *et alii*, 1957; Kelsall *et alii*, 1958) has shown that the titre of Rh antibodies in the maternal serum, when determined by the indirect antiglobulin method, is highly correlated with the severity of the hæmolytic process in the child. These studies show that an indirect antiglobulin titre at term of 1 : 64 or less is of no pathological significance. In such cases 100% survival occurs without treatment. It has been the practice in Perth for the last seven years, however, to exchange-transfuse most patients with a titre of 1 : 128, and all patients with a titre at term above this level.

As the indirect antiglobulin titre increases, so the severity of the disease in the infant becomes more severe; and even though the infant may be born alive and be exchange-transfused, the chance of survival diminishes. Thus, when the maternal indirect antiglobulin titre at term is 1 : 2048, a liveborn child has only a 50% chance of survival, under our methods of treatment, despite exchange-transfusion within the first few hours after birth (Kelsall *et alii*, 1957).

The majority of stillbirths due to Rh incompatibility are associated with maternal indirect antiglobulin titres of 1 : 1024, or higher. In 75% of these cases a high titre has persisted through at least the last ten weeks of pregnancy. For this reason, therefore, the indirect anti-globulin titre is a valuable guide in determining the necessity for the early induction of labour. If the mother's indirect antiglobulin titre at the thirtyfifth or thirtysixth week of pregnancy is rising toward, or is already at, 1 : 512, it is wise to consider inducing labour immediately (Kelsall *et alii*, 1958).

The indirect antiglobulin titre alone, however, may sometimes give rise to misleading predictions. In some cases an antiglobulin titre of 1 : 1024 may be associated with cord hæmo-globin values well within the normal range in the newborn infant. At the other extreme, the same titre may be associated with a moribund infant having a hæmoglobin of 4·0 grammes 100 millilitres and a reticulocyte count of 50%; or, even more disturbing, a mother may have an indirect antiglobulin titre of 1 : 1024 throughout pregnancy, only to be induced and delivered of an Rh-negative infant, whilst a similar serological picture in another case may

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result in a macerated stillborn fœtus at 35 weeks.

In an attempt to explain these discrepancies detailed studies in these laboratories revealed that if the newborn infant had no or little free Rh antibody in circulation, the case was always mild; whereas the most severe cases were associated with high indirect antiglobulin titres of free Rh antibody in the infant's serum. Attention was focussed, therefore, on the absorbing capacity of adult non-sensitized Rh-positive cells toward maternal sera from mild and severe cases. It was found that when one volume of maternal serum obtained on the day of delivery was incubated with one volume of adult Rh-positive cells, the amount of free antibody left behind in the serum was almost always identical with the amount found in one volume of the cord-serum of the infant. Further observations showed that it was unnecessary to use intact Rh-positive cells for carrying out this test, identical results being obtained if one volume of a suspension of hæmolyzed adult Rh-positive cells was substituted for the intact cells.

On the basis of these findings a new antibody test has been developed which, when used in conjunction with the indirect antiglobulin test, enables a much more accurate prognosis of the outcome of pregnancy to be made. This test is now referred to as the partial absorption (P.A.) test. The present paper details the technique of carrying out this new test. It discusses also the relationship of the P.A. test to other tests of Rh-antibody level currently in use, and outlines the behaviour of the P.A. titre during pregnancies which result in either Rh-negative infants or Rh-positive infants affected with hæmolytic disease. In the following paper (Kirk *et alii*, 1958) the prognostic value of the P.A. test is statistically evaluated for a series of 290 cases.

METHODS

Antibody Titrations

Indirect antiglobulin titrations were carried out using the technique described previously (Kelsall and Vos, 1952; Kelsall *et alii*, 1957). During the last year the technique has been improved by making use of a cell-washing machine to ensure standardization of the washing of the sensitized cells. This enables more uniform results to be obtained by different workers titrating the same serum. The details of the indirect antiglobulin titration using mechanical washing procedures have been described by Vos and Kirk (1958).

The blocking and conglutination tests were carried out by the method of Wiener and Hurst (1947) and Wiener, Hurst and Sonn (1947). Albumin titrations followed the procedure of Diamond and Denton (1945).

Preparation of Standard Hæmolysed Cell Suspensions

Heparinized or oxalated blood is collected from the arm veins of healthy Group O CDe/CDe (R_1R_1) or Group O CDe/cDE (R_1R_2) donors. The cells are packed and washed three times in large volumes of physiological saline, the samples are pooled, and the cells are finally packed by centrifuging at 3000 rpm for 30 minutes. An accurate 25% saline suspension of the packed cells is made, and this is divided into two millilitre samples in screw cap McCartney bottles ready for daily use. This standard suspension of cells is hæmolyzed by freezing to −20°C for 48 hours. The hæmolyzed cell suspension may be stored for at least 12 months at this temperature without detectable loss of absorbing capacity.

Procedure for the Partial Absorption Test

The standard hæmolyzed cell suspension is thawed to room temperature and shaken thoroughly before use. Then 0·4 mllilitre of this suspension is added to 0·4 millilitre of a 1 : 4 saline dilution of the maternal serum in a small test tube. The two solutions are mixed by shaking well and left at room temperature for 15 minutes. At the end of this time the free Rh antibody remaining in the mixture is titrated in doubling dilutions using the indirect antiglobulin technique. The first tube in this titration is read as 1 : 16, since this is the dilution at this stage of the original maternal serum. Partial absorption titres lower than 1 : 16 have been found to be of no significance, and are now scored as negative.

RESULTS

1. *The Partial Absorption Titre of the Maternal Serum and the Free Rh-Antibody in the Infant's Serum*

In Table I the P.A. titre of the maternal serum at the time of delivery is compared with the titre of free antibody in the infant's cord determined by the indirect antiglobulin method. A total of 187 cases is available in which the infant was liveborn Rh-positive and in which both titrations have been carried out. In all these cases the infant's cells gave a positive direct response to the Coombs test at the time of birth. For 166 of the cases (89%) the P.A. titre of the maternal serum was either identical with or within one tube dilution of the titre of free Rh antibody in the cord serum. In a

further 10 cases the two titration values did not differ by more than two dilution tubes.

Of the 11 cases shown in Table I, in which the maternal P.A. titre differs from the free antibody titre in the cord-serum by more than two dilution tubes, in one the mother was grossly œdematous, and in five the infant was œdematous at birth; simple dilution may account for part at least of the differences. In the four cases in which œdema was not present in the infant, we can offer no satisfactory explanation at present for the discrepant results.

Despite the wide range of clinical conditions in the infants belonging to this series, the P.A. titre of the maternal serum at the time of delivery in 90% of the cases is, within the limits of one tube dilution, the same as the free antibody titre of the infant's cord serum. This fact suggests that in the majority of cases the P.A. titre can be used during the course of pregnancy to indicate the amount of free antibody in the foetal circulation.

TABLE I.

Comparison of Maternal Partial Absorption Titre at the Time of Birth with Titre of Free Antibody in the Cord Serum

Tube Dilutions of Maternal Titre Compared with Cord-Serum Titre[1]	Number	Percentage
−3	1	0·5
−2	6	3·2
−1	29	15·5
0	109	58·3
+1	28	15·0
+2	4	2·1
+3	7	3·7
+4	3	1·6
All cases	187	99·9

[1] "0"=maternal P.A. titre identical with indirect antiglobulin titre of cord serum. "−1", "−2", "−3"=maternal P.A. titre one, two or three tube dilutions below indirect antiglobulin titre of cord serum. "+1", "+2", "+3", "+4"=maternal P.A. titre one, two, three or four tube dilutions above indirect antiglobulin titre of cord serum.

2. *Comparison of the Partial Absorption Test with Conglutination, Albumin, Blocking and Indirect Antiglobulin Tests*

Selected cases were chosen to compare the P.A. titre of the maternal serum with the blocking, conglutination, albumin and I.D.C. titre as determined by the standard techniques. The results for ten such cases are given in Table II. No correlation can be observed between the P.A. titre and the plasma conglutination or albumintitres. There is a correlation, however, between the P.A. titre and either the blocking titre or the indirect antiglobulin titre. These relationships have been explored more thoroughly on a larger series of cases. Table III gives the results for 42 samples of maternal sera that have been examined. There is a significant positive correlation between both P.A. titre and blocking titre, and P.A. titre and indirect antiglobulin titre, but the correlation coefficient ($r=0\cdot90$) for the former is slightly higher than that for the latter ($r=0\cdot83$).

TABLE II.

Comparative Tests on Maternal Anti-Rh Sera

Case Number	Partial Absorption Titre	Blocking Titre	Indirect Antiglobulin Titre	Plasma Conglutination Titre	Albumin Titre
302	1 : 2048	1 : 64	1 : 4096	1 : 16 TR	1 : 1024
213	1 : 1024	1 : 64	1 : 4096	1 : 64 TR	1 : 256
273	1 : 512	1 : 64	1 : 2048	1 : 256 TR	1 : 512
261	1 : 256	1 : 4	1 : 4096	1 : 1024	1 : 512
342	1 : 256	1 : 8	1 : 2048	1 : 128	1 : 512
267	1 : 256	1 : 4	1 : 1024	1 : 512	1 : 4096
231	Negative	Negative	1 : 512	1 : 1024	1 : 2048
278	Negative	Negative	1 : 512	1 : 256	1 : 4096
274	Negative	Negative	1 : 256	1 : 64	1 : 2048
340	1 : 8	Negative	1 : 128	1 : 1024	1 : 512

The other significant observation which can be made is that, although the P.A. titre is correlated with the blocking titre, the method is more sensitive than that of the blocking test. Thus in some cases indirect P.A. titres as high as 1 : 256 were recorded even though the result of the blocking test was still negative. In this series of cases the highest blocking titre observed was 1 : 128.

3. *Behaviour of the Partial Absorption Titre During Pregnancy*

Examination of detailed case-histories in which the maternal P.A. titre has been determined at regular intervals throughout pregnancy reveals certain basic patterns in the P.A. titre response associated with clinical outcome ranging from mildly affected infants to grossly hydropic stillborn infants. Since the pattern of response is sometimes more informative than a single titration value obtained near term, typical examples are outlined below.

(*a*) *Stillbirths.*—In nearly all cases of stillbirth due to Rh-incompatibility the indirect antiglobulin titre at the time of delivery is 1 : 1024 or higher, and has been maintained at a high level for at least 10 weeks before delivery (Kelsall *et alii*, 1958). The P.A. titre in these cases can remain at a relatively low level throughout the greater part of pregnancy, but may then rise rapidly. If death occurs *in utero*, the P.A. titre may start to fall again, though the indirect antiglobulin titre generally remains

TABLE III.

Partial Absorption Titre, Blocking Titre and Indirect Anti-Globulin Titre for Various Maternal Sera

Number	Partial Absorption Titre	Blocking Titre	Indirect Antiglobulin Titre	Number	Partial Absorption Titre	Blocking Titre	Indirect Antiglobulin Titre
1	Negative	Negative	1 : 512	22	1 : 32	Negative	1 : 512
2	Negative	Negative	1 : 512	23	1 : 256	Negative	1 : 4096
3	Negative	Negative	1 : 128	24	1 : 64	Negative	1 : 256
4	Negative	Negative	1 : 256	25	1 : 128	Negative	1 : 2048
5	Negative	Negative	1 : 128	26	1 : 128	1 : 2	1 : 1024
6	1 : 16	Negative	1 : 512	27	1 : 256	1 : 4	1 : 4096
7	1 : 8	Negative	1 : 128	28	1 : 256	1 : 4	1 : 1024
8	1 : 8	Negative	1 : 512	29	1 : 256	1 : 4	1 : 1024
9	1 : 16	Negative	1 : 1024	30	1 : 256	1 : 4	1 : 2048
10	1 : 4	Negative	1 : 256	31	1 : 128	1 : 4	1 : 512
11	1 : 32	Negative	1 : 512	32	1 : 256	1 : 8	1 : 2048
12	1 : 16	Negative	1 : 256	33	1 : 512	1 : 16	1 : 2048
13	1 : 8	Negative	1 : 512	34	1 : 256	1 : 16	1 : 4096
14	1 : 4	Negative	1 : 512	35	1 : 512	1 : 16	1 : 1024
15	1 : 4	Negative	1 : 128	36	1 : 512	1 : 32	1 : 2048
16	1 : 4	Negative	1 : 512	37	1 : 1024	1 : 32	1 : 2048
17	1 : 32	Negative	1 : 512	38	1 : 2048	1 : 64	1 : 4096
18	1 : 16	Negative	1 : 512	39	1 : 1024	1 : 64	1 : 4096
19	1 : 32	Negative	1 : 1024	40	1 : 512	1 : 64	1 : 2048
20	1 : 64	Negative	1 : 2048	41	1 : 1024	1 : 64	1 : 4096
21	1 : 32	Negative	1 : 128	42	1 : 4096	1 : 128	1 : 8192

steady (*vide infra*). A high indirect antiglobulin titre maintained throughout pregnancy does not necessarily mean that the fœtus will be seriously affected. But if a high indirect antiglobulin titre is associated with a high P.A. titre throughout pregnancy and the fœtus is Rh-positive, the outcome is invariably fatal.

(*b*) *Severely Affected Liveborn Infants.*—These are nearly always associated with a maternal indirect antiglobulin titre at term of 1 : 1024 or higher, but the P.A. titre is often low early in pregnancy and shows a sudden rise, often to a high level shortly before birth.

(*c*) *Moderately Affected Infants.*—One of the puzzling features of previous work on the relationship between maternal antibody levels and severity of the disease in the infant has been the occurrence of relatively high antibody levels in the maternal serum, sometimes throughout pregnancy, in cases which have terminated with the birth of a moderately affected infant. In typical cases the indirect antiglobulin titre of the maternal serum may persist at a moderately high level of 1 : 512 throughout the greater part of pregnancy, but the P.A. titre remains low, at 1 : 16 or less, and does not rise appreciably near term. Another category of moderately affected infants can be distinguished in those cases in which the indirect antiglobulin titre is low or zero at the commencement of pregnancy, but rises to a high value during the third trimester. The P.A. titre may show a parallel rise. In these cases, however, the rise in antibody level occurs too late for serious damage to the infant to occur before birth.

(*d*) *Rh-Negative Infants.*—In families in which the father is heterozygous D/d, it is important to be able to distinguish an Rh-negative fœtus from an Rh-positive fœtus if a correct prognosis is to be made before birth. Unfortunately, there is no certain way at present by which this may be done. The behaviour of the P.A. titre during pregnancy, however, is a somewhat better guide than the behaviour of the indirect antiglobulin titre alone. Cases in which the indirect antiglobulin titre, because of antibodies from a previous pregnancy, remains high through the last four months, but the P.A. titre remains negative or very low, suggest that no fresh immunization has been taking place. In general the P.A. titre responds more rapidly than the indirect antiglobulin titre to a new episode of immunization, and it tends to fall again to low levels before the next pregnancy commences. Persistent low P.A. titres throughout pregnancy, therefore, are more frequent where the fœtus is Rh-negative and is failing to stimulate the production of more Rh-antibody.

(*e*) *The Effect of Death in Utero and Maternal Oedema on the P.A. titre.*—In some cases a fall in the P.A. titre may occur as pregnancy progresses. This may be due to three causes: (*a*) an Rh-negative fœtus, (*b*) death *in utero* and (*c*) maternal œdema. Both (*b*) and (*c*) indicate the remarkable sensitivity of the P.A. titre as an index of what is occurring in the mother and the fœtus. Sometimes, in severe cases of Rh-immunization, the fœtal heart stops several weeks before parturition takes place. If the P.A. titre is high when death occurs, it shows a fall in about one half of the cases. In a series of 20 stillbirth cases in which the P.A. titre has been determined at regular intervals through the last ten weeks of pregnancy, eight cases showed a

marked decline of two or more dilution tubes in the P.A. titre before the end of pregnancy. In each of these cases parturition resulted in the presentation of a badly macerated fœtus. In six of the remaining 12 cases a macerated fœtus also presented, but there was no detectable fall in the P.A. titre before delivery.

It is important to note that, although the P.A. titre falls in nearly half these 20 stillbirth cases, in no single case does the indirect antiglobulin titre show a fall; indeed, in one case there was an increase in the indirect antiglobulin titre from 1 : 256 to 1 : 1024 at the same time as the P.A. titre fell from 1 : 128 to 1 : 32.

Discussion

A number of attempts has been made to analyse the complexity of the maternal antibody response to Rh antigens by both physico-chemical and serological means (Mohn and Witebsky, 1948; Hill *et alii*, 1949; Cann *et alii*, 1952; Krieger and Williams, 1955; Wiener and Gordon, 1953; Greenwalt and Wagner, 1955), but so far no one has evolved a relatively simple test which, when applied to the maternal serum antenatally, can be used to predict with high probability the severity of the hæmolytic process in the child and its chances either of being stillborn or, if born alive, of living with or without the use of exchange transfusions.

It has been maintained previously (Davidsohn and Stern, 1948; Potter, 1948; Wiener *et alii*, 1952) that blocking antibodies are associated with more severe manifestations of erythroblastosis in the baby, and de Kromme and Vervaat (1953) have attempted to show that variations in conglutination-positive and conglutination-negative reactions of the maternal serum can be used to explain differences in the severity of the disease.

The titration values obtained in the P.A. test are of interest, therefore, in attempting to understand the complexity of Rh-antibody production in response to the stimulus of an incompatible pregnancy. Ten years ago Diamond noted that severe cases of erythroblastosis were associated with the presence of free Rh antibody in the cord serum of the affected infant (Diamond, 1947). The present study shows that, if one volume of packed adult cells or the equivalent volume of hæmolyzed cells is used to absorb one volume of maternal serum, the antibody left free in the serum, as measured by the indirect antiglobulin technique, can vary over a wide range of values. For instance, in cases in which the indirect antiglobulin titre is 1 : 1024, the antibody left free in the serum can vary from zero up to, but never exceeding, 1 : 1024. The titre of free antibody is the P.A. titre, and the present results therefore confirm and extend the earlier observations of Diamond.

The first conclusion to be drawn from these results is that *in vivo* the absorbing power of the fœtal red cells is roughly the same as that of adult red cells. Secondly, since all these cases in which the P.A. titre is zero are mildly affected, it would suggest that the hæmolytic process in the fœtus does not begin to take place rapidly until all the antigenic sites on the red cell have been loaded with antibody, or blocked from accepting more. Work already in progress in this laboratory indicates that at least two kinds of antigen-antibody reaction are involved in the process of absorbing Rh antibodies on Rh-positive cells. In the first of these the antibody is bound firmly to the cell and can be released only by extracting with ether and completely disrupting the structure of the cell surface. In the second the antibody bound to the cell can be released simply by freezing the cell and thawing it. In this way the cell is hæmolyzed, but the antigenic sites are relatively undamaged. When cells are sensitized with an Rh serum with a zero P.A. titre and then thoroughly washed, no Rh antibody is released after hæmolysis caused by freezing and thawing. If, however, the cells are sensitized with an Rh serum which has a P.A. titre, the titre of antibodies released from the cells after freezing and thawing is found to be identical with the P.A. titre of the original serum.

These preliminary results suggest that under conditions where the sensitizing serum has a positive P.A. titre, a portion of the Rh antibodies is in equilibrium between the cell and the serum. A tentative hypothesis suggests that it is only when sensitized cells carry antibody in this manner that they are hæmolysed readily in the fœtal circulation. If this interpretation is correct, then the amount of free antibody in the cord serum, as estimated by the P.A. technique, would be related directly to the rate of cell destruction *in vivo*. The persistence of such a process at a high rate over several weeks is almost certain to result in severe or fatal manifestations of erythroblastosis in the infant. A fuller account of the relationship between the antibody free in the serum and that bound to the cell will be published in due course.

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THE EVALUATION OF THE PARTIAL ABSORPTION TEST FOR RH ANTIBODIES IN THE PROGNOSIS OF HÆMOLYTIC DISEASE OF THE NEWBORN[1]

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SUMMARY

The partial absorption test for titrating Rh antibodies in the maternal serum has been evaluated as a prognostic tool in a series of 290 cases of infants born to Rh-immunized women. The P.A. test enables a more accurate prognosis to be made of the outcome for the infant than if the indirect antiglobulin titre of the maternal serum is used alone.

The statistical coefficient of correlation between the P.A. titre of the maternal serum at the time of birth and cord blood hæmoglobin for liveborn Rh-positive infants is −0·72.

The P.A. titre is unreliable in predicting stillbirths unless regular titrations have been carried out at least from the twentyfourth week of pregnancy. The unreliability is due to the rapid fall in P.A. titre in many cases after the foetus has died *in utero*. However, if the P.A. titre at the end of the second trimester is 1 : 64 or higher, the outcome is almost invariably fatal.

The use of the P.A. test in conjunction with the ordinary indirect antiglobulin titrations of the maternal serum enables a more accurate discrimination to be made between mild and severe cases of hæmolytic disease of the newborn. It also makes possible the more reliable diagnosis of an Rh-negative infant in cases in which the father is known to be heterozygous D/d, and is a valuable guide for giving advice to parents of Rh-affected infants on when to start another pregnancy.

It is recommended that exchange-transfusion should be carried out in all cases in which the maternal indirect antiglobulin titre at the time of birth is 1 : 256 or higher. Exchange-transfusion is not warranted if the maternal indirect antiglobulin titre is less than 1 : 128 at the time of birth. Where the maternal indirect antiglobulin titre is 1 : 128, exchange-transfusion is not necessary if the P.A. titre is zero.

IN a recent publication (Kelsall, *et alii*, 1957) we have evaluated the prognostic significance of the titre of Rh antibodies in the maternal serum at term when determined by a standardized indirect antiglobulin technique.

In a subsequent study Kelsall, *et alii* (1958) have shown that the prognostic value of the maternal antiglobulin titre can be considerably improved if titrations are carried out regularly during the last ten weeks of pregnancy. The titration values are given a code number from 1 to 9, and these are summed for each week to give a titre index. This is a measure not only of the height of the titre, but of the length of time that the titre has been operating. Although the titre index is a much better guide to the likely outcome of any particular pregnancy than is a single titre value for antibodies in the maternal serum at term, there is still a small number of cases which do not fit into the general pattern. We believe that the explanation for some, at least, of these exceptional cases is to be found in the differing amounts of antibody which are left free in the infant's serum. In the preceding paper, Vos (1958) has described a simple partial absorption (P.A.) test which enables an estimate of the amount of antibody free in the foetal serum to be made from titrations carried out on the maternal serum. The present paper provides a more rigorous evaluation of this test.

METHODS

Venous blood samples were collected from all Rh-negative women as part of the routine ante-natal tests. When Rh antibodies were present in the maternal serum every effort was made to obtain samples at regular intervals. During the last three months of pregnancy samples were taken at fortnightly or even weekly intervals whenever possible. Samples of infant blood at the time of birth were obtained

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from the foetal end of the umbilical cord after section. The indirect antiglobulin procedure used in this laboratory for titrating Rh antibodies has been described previously (Kelsall *et alii*, 1957). More recently a mechanical cell-washing machine has been used for washing the sensitized cells. This ensures greater uniformity of results between different workers (Vos and Kirk, 1958), a development which is of great significance if titration values are to be used for prognostic purposes. The method of carrying out the partial absorption test on maternal serum has been described in the preceding paper (Vos, 1958).

The determination of cord-blood hæmoglobin values and reticulocyte percentages, and the methods of carrying out exchange-transfusion have been described in detail by Kelsall *et alii* (1957).

RESULTS

From July, 1953, to April, 1957, we have had 290 cases of Rh-immunized mothers for whom the indirect antiglobulin titre and the P.A. titre of the maternal serum at the time of delivery were available. There were 41 liveborn Rh-negative infants. Of the remaining 249 Rh-incompatible pregnancies, 36 resulted in stillborn infants: of the 213 liveborn Rh-positive infants 21 subsequently died, despite prompt and adequate exchange-transfusion in all cases in which the infant was not moribund at the time of birth.

Maternal P.A. Titre at Delivery and Survival of Rh-Incompatible Infants

The single fatality among the 57 cases in which the P.A. titre at the time of delivery was zero was an anencephalic monster (see Table I). There were no pathological signs that death in this case was in any way due to Rh incompatibility. In addition, one of the neonatal deaths, in which the mother had a P.A. titre of 1 : 16, was due primarily to obstruction of the duodenum. Of the other fatalities in the low-titre categories 1 : 16 and 1 : 32 shown in Table I, at least one of the neonatal deaths and both stillbirths were to mothers who were suffering from toxæmia of pregnancy with marked œdema. In many cases œdema associated with a stillbirth results in a fall in the P.A. titre before birth (Vos, 1958), and this may well have happened also in the other case where the infant died very soon after birth. The fall in the P.A. titre reduces its usefulness as a prognostic guide in such cases of course if the only titration carried out is near the end of pregnancy.

Bearing these points in mind it can be concluded that the risk of stillbirth due primarily to Rh incompatibility (when the maternal P.A. titre at birth is lower than 1 : 64) in the absence of marked œdema in the mother is no greater than in normal pregnancies. Similarly, in the absence of maternal œdema only 1% to 2% of the liveborn incompatible infants will subsequently die if the maternal P.A. titre at birth is less than 1 : 64. For P.A. titres of 1 : 64 or higher, however, the risk of neonatal death or stillbirth rises sharply. In two-thirds of the cases with a maternal P.A. titre at birth of 1 : 1024, the infants either were stillborn or died soon after birth, whilst no infant has survived yet when the maternal P.A. titre was 1 : 2048.

TABLE I.

Distribution and Outcome of Cases of Rh-Incompatible Infants Born to Immunized Mothers by Partial Absorption Titre of the Maternal Serum at Term.

P.A. Titre	Liveborn Infants		Stillborn Infants	Total Infants	Percentage Mortality
	Survived	Died			
0	56	0	1	57	1·75
1 : 16	40	3	1	44	9·09
1 : 32	17	2	1	20	15·00
1 : 64	25	2	3	30	16·67
1 : 128	10	2	3	15	33·33
1 : 256	25	2	7	34	26·47
1 : 512	12	5	8	25	52·00
1 : 1024	7	4	10	21	66·67
1 : 2048 or higher	0	1	2	3	100·00
All titres	192	21	36	249	22·89

Correlation of Maternal P.A. Titre with Cord-Blood Hæmoglobin Values

It has been shown previously (Kelsall *et alii*, 1957) that the maternal indirect antiglobulin titre at term is highly correlated with cord-blood hæmoglobin values. The relationship between the two is expressed by the correlation coefficient $r = -0{\cdot}62$. For 178 of the cases of liveborn Rh-incompatible infants in the present series, both cord-blood hæmoglobin values and the P.A. titre of the maternal serum at term are available. The distribution of these values, together with the method of treatment and outcome, is shown in Figure I. If this distribution is compared with the one published previously for maternal indirect antiglobulin titre at term and cord-blood hæmoglobin value (Kelsall *et alii*, 1957), it is clear that the P.A. titre of the maternal titre gives a much better indication of the severity of the hæmolytic process in the infant

than does the maternal indirect antiglobulin titre. This is in fact born out by the statistical analysis, which gives a correlation coefficient between the P.A. titre and cord-blood hæmoglobin value of $r = -0{\cdot}72$.

For infants compatible with their mothers for the Rh factor, the range of cord-blood hæmoglobin values is from 11·0 to over 20·0 grammes per 100 millilitres, with a mean value of 16·24 and a standard deviation of 1·7 (Kelsall *et alii*, 1957). It might be expected, therefore, that for any particular level of the maternal P.A. titre there would be a corresponding spread of cord-blood hæmoglobin values. Figure I shows this to be true.

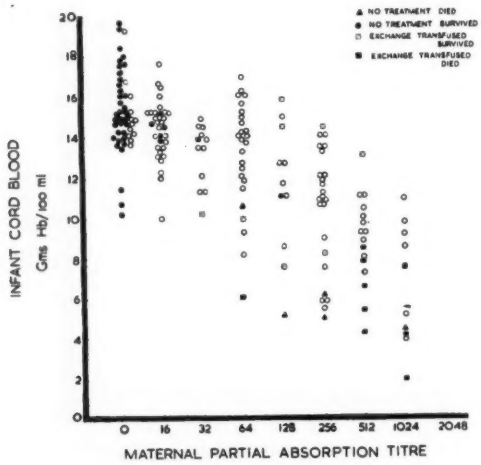

FIGURE I

The distribution of 179 live-born Rh-incompatible infants by cord-blood hæmoglobin value and maternal P.A. titre at the time of delivery

Relationship between Maternal Indirect Anti-globulin Titre and Partial Absorption Titre

If all cases are considered in which the maternal indirect anti-globulin titre at the time of birth is 1 : 1024, the cord-blood hæmoglobin values can range from 3·0 to 17·0 grammes per 100 millilitres, in approximately half of the cases being above 10·0 grammes per 100 millilitres (Kelsall *et alii*, 1957). In our experience the infants in these latter cases are not bad risks for exchange-transfusion. However, in cases in which the maternal indirect antiglobulin titre at birth is 1 : 1024, but the cord-blood hæmoglobin value is below 10·0 grammes per 100 millilitres, the infants have a much poorer prognosis for survival even when exchange-transfused.

It became clear early in the present study that, for any given maternal indirect antiglobulin titre, the P.A. titres of the maternal serum can vary over a wide range in different cases. In Table II the distribution of cord-blood hæmoglobin values for 95 Rh-positive liveborn infants, born to mothers with a maternal indirect antiglobulin titre at term of 1 : 512 to 1 : 4096, is given in relationship to the difference between the indirect antiglobulin titre and the P.A. titre of the maternal serum. The difference in titre is expressed in dilution tube numbers. This means that, for a particular indirect antiglobulin titre (1 : 1024, for example), the corresponding P.A. titre can range from the same value (difference=0) down to 1 : 16 (difference=6 dilution tubes).

From the distribution of cases in Table II it is clear that there is a high correlation between the difference in titre and the cord-blood hæmoglobin value ($r = 0{\cdot}61$). This means that the P.A. titre enables us to improve considerably on the prognosis based on the maternal indirect antiglobulin titre at term. Of the 95 liveborn infants recorded in Table II, 15 subsequently died. None of these deaths occurred, however, among the 24 infants in whom the P.A. titre was more than three dilution tubes lower than the maternal indirect antiglobulin titre at term. Thus, a high indirect antiglobulin titre in the mother need not be considered a serious sign if the P.A. titre at the same time is low.

TABLE II.

Relationship between Cord-Blood Hæmoglobin Values in Liveborn Rh-Incompatible Infants and the Difference between the Maternal Indirect Antiglobulin Titre and P.A. Titre at Term.

Cord-Blood Hæmoglobin Value (Grammes per 100 Millilitres)	Difference in Tube Numbers between Maternal Indirect Anti-Globulin Titre and P.A. Titre							All Titre Differences
	6	5	4	3	2	1	0	
18·0 and over ..	—	—	—	—	—	—	—	—
16·0 to 17·9 ..	1	—	—	—	—	—	—	1
14·0 to 15·9 ..	1	6	4	6	3	—	—	20
12·0 to 13·9 ..	2	3	3	4	4	3	—	19
10·0 to 11·9 ..	—	—	4	4	10	4	—	22
8·0 to 9·9 ..	—	—	—	3	7	4	1	15
6·0 to 7·9 ..	—	—	—	1	5	2	0	8
4·0 to 5·9 ..	—	—	—	1	3	4	1	9
2·0 to 3·9 ..	—	—	—	—	—	1	0	1
All values ..	4	9	11	19	32	16	2	95

Stillbirths

There were 36 stillbirths among the 249 incompatible infants born in the present series. One of these was an anencephalic monster: the maternal indirect antiglobulin titre was only 1 : 128, and the P.A. titre was zero at term, so that it seems reasonable to consider that Rh incompatibility was not of ætiological significance. Of the remaining 35, 27 were associated with peak P.A. titres of 1 : 256 or above (see Table I). It is necessary to use the peak P.A. titre in these cases, since in many cases of stillbirth the P.A. titre drops rapidly after death has occurred *in utero*. A terminal value of the P.A. titre in such cases therefore does not represent the true value to which the living fœtus has been exposed.

The rapid drop in the maternal P.A. titre which occurs in many instances after uterine death has taken place means that prognosis based on a single titration value obtained near term is likely to be misleading. This probably explains why eight of the stillbirths in Table I were associated with P.A. titres of 1 : 128 or less. In a number of these it was possible to obtain blood samples from the mother only near term. If these cases had been titrated at regular intervals over at least the last ten weeks of pregnancy, the peak values obtained would almost certainly have been significantly higher.

For 73 of the incompatible pregnancies in the present series antibody titrations have been carried out on the maternal serum before the end of the second trimester. Table III shows that in these cases, when the P.A. titre was already 1 : 64 or higher at the twentyfourth week of gestation, only one out of 17 infants survived; 12 were stillborn, and four died after birth. Indeed, of the children of mothers who had a P.A. titre at all at the end of the second trimester, only 14 out of 43 incompatible infants survived.

Clearly the occurrence of a P.A. titre in the maternal serum at any level early during pregnancy is an unfavourable sign. If it is 1 : 64 or higher, the outcome is almost invariably fatal.

The Rh-Negative Infant

There were 41 Rh-negative infants born to mothers who had been immunized by a previous pregnancy. In the majority of these cases the indirect antiglobulin titre was of little value in helping to predict before birth whether the infant would be Rh-positive or Rh-negative. In fact, 34 of these Rh-negative infants were born to mothers with an indirect antiglobulin titre of 1 : 128 or higher—that is, in the range which would make an exchange-transfusion necessary if the infant was Rh-positive (Table IV). Actually, 25 of the pregnancies were terminated artificially, since a correct differential diagnosis of the infant's genotype could not be made before birth.

TABLE IV.

Distribution of Rh-Negative Infants Born to Immunized Mothers by Indirect Antiglobulin Titre and P.A. Titre of the Maternal Serum at Birth

Titre	Distribution of Infants	
	P.A. Titre	Indirect Anti-globulin Titre
Zero	18	—
1 : 16	13	3
1 : 32	4	2
1 : 64	3	2
1 : 128	3	11
1 : 256	—	9
1 : 512	—	11
1 : 1024	—	3
Total	41	41

The P.A. titres of the same cases shows an entirely different distribution; 18 of the mothers had a zero P.A. titre at the time of birth, and a

TABLE III.

Outcome of Cases in which the Maternal P.A. Titre was Determined before the End of the Second Trimester

Outcome	Maternal P.A. Titre at or Before 24 Weeks' Gestation									
	0	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048	All Titres
Liveborn—survived	28	7	6	1	0	0	0	0	0	42
Liveborn—died ..	2	1	2	3	1	0	0	0	0	9
Stillborn	2	3	5	1	2	5	1	2	1	22

further 13 had a P.A. titre only in the lowest dilution. In these latter cases the P.A. titre value of 1 : 16 had been maintained unchanged during the greater part of pregnancy. The constant antibody level was true also of all but two of the 10 cases with a maternal P.A. titre higher than 1 : 16. In one of the exceptional cases, the P.A. titre fell from an early value of 1 : 512 to 1 : 128 at term. In the other case, the P.A. titre rose from 1 : 64 to 1 : 128. This is unique in our experience and represents the only example in which an Rh-negative infant was born to a mother who had a rise in either indirect antiglobulin or P.A. titre during the course of pregnancy.

The distribution of cases shown in Table IV shows that the P.A. titre is of a greater value than the maternal indirect antiglobulin titre in deciding whether a pregnancy should be allowed to terminate spontaneously. If the father is known to be heterozygous D/d, than a zero or constant low P.A. titre maintained throughout pregnancy is a reasonable indication that the infant is Rh-negative. In such cases, even if the indirect antiglobulin titre is high, the pregnancy can be allowed to continue. It must be emphasized, however, that if this course is adopted, weekly checks of the P.A. titre are essential during the last two months. In some cases in which an incompatible infant is involved, the P.A. titre may remain at a low level until the last few weeks, only to rise rapidly, with serious consequences for the infant if labour is not induced immediately.

DISCUSSION

The technique developed in these laboratories for estimating indirectly the amount of Rh antibody free in the infant's serum by a partial absorption test on the maternal serum (Vos, 1958) gave early indications that it would improve the reliability of prognosis based on the maternal indirect antiglobulin titre alone. Our present evaluation of this test, based on a series of 290 cases during the last four years, has confirmed this earlier hope.

The correlation between cord-blood hæmoglobin value and maternal P.A. titre is higher than between cord-blood hæmoglobin value and the ordinary indirect antiglobulin titre. Indeed, the variance in cord-blood hæmoglobin value for any particular maternal indirect antiglobulin titre is due almost entirely to the variance in the P.A. titre. The value of the discrimination between mild and severe cases which the use of the P.A. test makes possible is indicated by the fact that no deaths have occurred among cases with a maternal indirect antiglobulin titre of 1 : 512 or higher where the maternal P.A. titre was more than three dilution tubes lower (see Table II).

The P.A. titre is useful also in discriminating between the need for exchange-transfusion in mildly affected infants. The practice in this hospital originally was to exchange-transfuse all infants born to mothers with an indirect antiglobulin titre of 1 : 128 or higher, leaving untreated those with an indirect antiglobulin titre of 1 : 64 or less. However, there are many cases with borderline values of 1 : 128 in which the infant is born with a cord-blood hæmoglobin value well within the normal range, with no elevation of the reticulocyte levels and no evident bilirubinæmia. In these cases the infants almost always have a zero P.A. titre, and during the last two years they have been untreated. Despite transient jaundice, which sometimes becomes moderately intense, they have all survived and are now normal in all respects.

The use of the P.A. test enables also a much safer prediction of an Rh-negative infant in cases in which the father is known to be heterozygous D/d. This is an important use of the test if it means that some pregnancies which would otherwise merit early induction will be allowed to continue to full term when the P.A. titre is zero or steady at 1 : 16.

However, the P.A. test is not so valuable in predicting stillbirths unless the titrations have been carried out at regular intervals during pregnancy. This appears to be due mainly to the very marked drop in the P.A. titre which may take place when death occurs *in utero*. Terminal titration values obtained shortly before birth therefore mislead, since they may suggest a milder degree of involvement than is true when the pregnancy terminates with the birth of a grossly hydropic fœtus. However, our records show that, if the maternal P.A. titre at the end of the second trimester is 1 : 64 or higher, the outcome is almost invariably fatal. So far only one case out of 17 for which we have the relevant information has survived.

The foregoing discussion indicates another important use of the P.A. test. We are approached frequently by women who have had one or more Rh-affected infants and who wish to know if it is wise to start another pregnancy. In the past we have used the ordinary indirect antiglobulin titre of the womens' serum at the time as a guide to the advice given ; our practice has been to discourage pregnancy if the indirect antiglobulin titre is higher than 1 : 128. A further discrimination can now be made on the

basis of the P.A. titre. Where the P.A. titre is 1 : 64 or higher, pregnancy is certainly contra-indicated, for the chances of a successful outcome are negligible. Indeed, in cases in which the husband is homozygous D/D, it is inadvisable to start another pregnancy if the P.A. titre is 1 : 16 or higher.

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